January 1961
VOLUME 83/NUMBER 1

THE AMERICAN REVIEW OF Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN THORACIC SOCIETY
Medical Section of the National Tuberculosis Association

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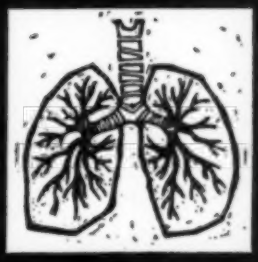

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VOLUME 83/NUMBER 1

THE AMERICAN REVIEW OF Respiratory Diseases

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

CONTENTS

The So-called Problem of Unclassified Mycobacteria. A Review. C. Xalabarder 1
Dilated Bronchial Mucous Glands in Chronic Bronchitis, a Neglected Morphologic Finding. Correlation of Bronchoscopic and Bronchographic Appearance. John H. Hirschfeld 16
The Diagnosis of Acute Pulmonary Embolism by Arteriography. William S. Stoney and Jesse E. Adams 26
Bronchial Arterial Collateral Circulation. Effect of Experimental Ligation of the Pulmonary Artery and Subsequent Reanastomosis. Ralph D. Alley, L. H. S. Van Mierop, Allan S. Peck, Harvey W. Kausel, and Allan Stranahan 31
A Study of Viruses Implicated in Acute Respiratory Disease in Chicago from October, 1958, to June, 1959. Dorothy Hamre, Joyce C. Lashof, J. Allen Marshall, James E. Cassidy, Mabel Smith, and C. Robert Bennett 38
Leukocytotoxic Component of Pine Pollen. Effect on Peripheral Blood Leukocytes of Normal and Tuberculin-hypersensitive Guinea Pigs. Robert A. Patnode and Bernard W. Janicki 43
An Evaluation of Chemotherapy in Pulmonary Tuberculosis. High Doses of Isoniazid plus PAS versus Low Doses of Isoniazid plus PAS. Stephen J. Berte and William G. Dunnington 50
Specificity of Mycobacterial Sensitins. I. Studies in Guinea Pigs with Purified "Tuberculin" Prepared from Mammalian and Avian Tubercle Bacilli, *Mycobacterium Balnei*, and Other Acid-fast Bacilli. Mogens Magnusson 57
Specificity of Mycobacterial Sensitins. II. Studies in Guinea Pigs with Purified Sensitin Prepared from Unclassified Acid-fast Bacilli. M. Magnusson, H. C. Engbaek, and M. Weis Bentzon 69
Effects of Killed Tubercle Bacilli (BCG) and Constituents upon the Reticuloendothelial System, the Internal Organs, and the Distribution of Circulating Leukocytes of Normal Albino Mice. Claude A. Bouvier and Diethelm Böhme 85
The Diagnostic Reliability of Acid-fast Bacilli Demonstrated in Aspirated Gastric Contents. Philip C. Pratt and Robert J. Atwell 96
Studies on *Mycobacterium Tuberculosis* H37Rv Grown *in Vivo*. Michael Artman and Adam Bekierkunst 100
Case Reports:
Cavernostomy. A Salvage Procedure for Giant Tuberculous Cavities. J. N. Briggs, N. C. Hamel, and R. R. Torrey 107
Claviculectomy in the Management of Post-resectional Space Problems. Jesse P. Chapman, Jr. 110
Chest Conference. University of California School of Medicine 113
Notes:
Studies in Sulfur Metabolism of *Mycobacterium Tuberculosis*, H37Rv Strain, and Development of a Synthetic Medium Designated as "M-Medium." S. L. Mukherjee *et al.* 117
Synergistic Combinations of Penicillin and Isoniazid *in Vitro* against *M. Smegmatis*. E. K. M. Vaichulis 121
An Improved Breath-actuated Bellows Apparatus for the Determination of Forced Expiratory Capacities. M. J. Small and S. Abramowitz 124
The Surgical Treatment of Patients with Infections of the Lung Due to Unclassified Acid-fast Bacilli. A. J. Taylor 127
Correspondence: Sickle Cell Trait and Tuberculosis. S. Katz and P. McCurdy 130
Books 131
American Thoracic Society:
Organization and Committee Structure, 1960–1961 134
Notices 145
Abstracts 147

ANNUAL MEETING, CINCINNATI, OHIO, May 21–25, 1961

See page 4 of advertisements

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Membership: All physicians and surgeons in the United States, Canada, and other countries who are interested in tuberculosis and other respiratory diseases may apply for membership in the American Thoracic Society. The dues for active membership in the Society are $18 per year, which includes membership in the National Tuberculosis Association, copies of the Society's News Letter, and a subscription to the REVIEW and to the monthly BULLETIN of the National Tuberculosis Association.

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MANUSCRIPTS

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

Manuscripts should be sent in duplicate to the office of the Editor, Dr. Walsh McDermott, 1790 Broadway, New York 19, N. Y., mailed flat and transmitted by first-class mail with postage for return if not acceptable. They should be in English, double-spaced, typewritten on one side of the page with wide margins. Tabular data should not be included in the text but should be presented in separate tables with appropriate reference in the text. All cases of pulmonary tuberculosis should be classified according to the 1955 edition of *Diagnostic Standards and Classification of Tuberculosis* published by the National Tuberculosis Association. All case reports should be written in complete sentences. Authors should exercise particular care in the preparation, notation, and description of figures and tables. The form of reference used in the REVIEW should be carefully followed and references should be checked for accuracy. Each table must be accompanied by a descriptive title, and a suitable legend must be provided for each figure. Drawings must be made with india ink and all lettering should be made with a stencil. The quality of the reproduction of a roentgenogram depends upon both the quality of the original picture and of the glossy print obtained from photographing that picture. If a contributor does not have facilities available, preparation of figures or reproduction of roentgenograms may be handled through the REVIEW office. In those circumstances, the contributor should supply a neat, properly proportioned, and suitably entitled drawing or the original roentgenogram, and will be expected to defray the cost of these services.

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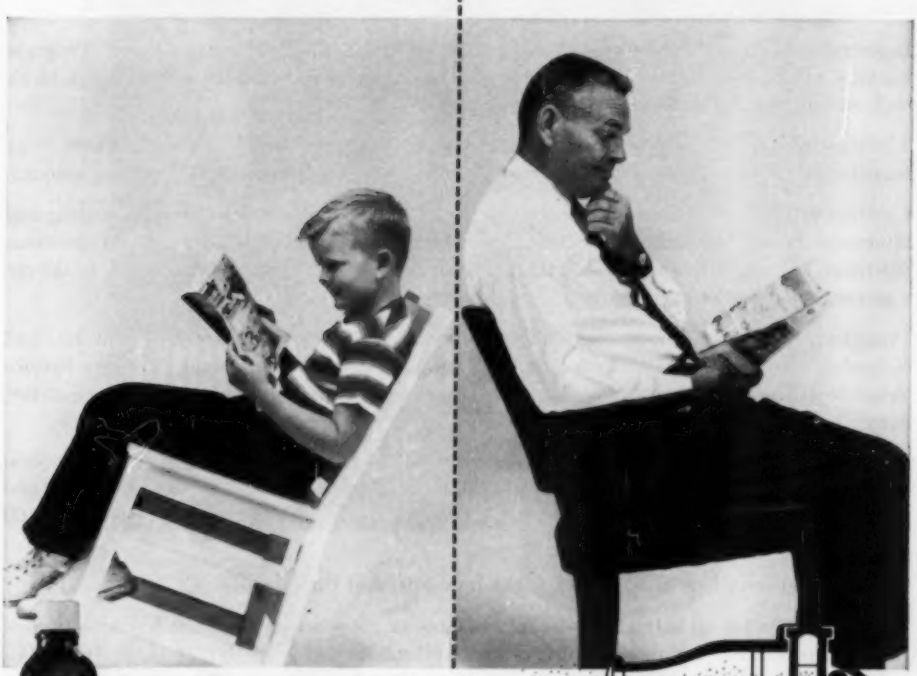

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Registration and the exhibits program will be in the Netherland Hilton Hotel. Program sessions will be held in the Netherland Hilton and cooperating hotels, each within a block of Fountain Square in the heart of the city.

The registration desk will be open beginning at 10:00 a.m., Sunday, May 21. There is no registration fee. A registration badge must be worn to ensure admission to program sessions.

Exhibits will open at 8:30 a.m., Monday, May 22, and will be on view, during morning and afternoon hours, throughout the meeting. The high caliber of exhibits, as at previous meetings, will make this a phase of the program to which all attending will want to devote a generous portion of their time.

Preceding the general meetings—ATS Committee and Council meetings will be held Saturday and Sunday, May 20 and 21. The annual Tuberculosis Nursing Advisory Service Seminar will be held on Saturday, May 20. There will be special interest sessions on Sunday, May 21.

Medical, public health, and nursing sessions will begin on Monday, May 22. General sessions will include an opening program Monday at 9:00 a.m. A keynote, awards, and NTA business session will be held Monday evening, and a closing general session at 11:00 a.m., Thursday, May 25.

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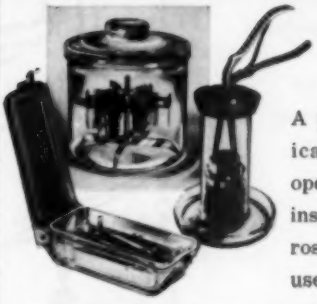

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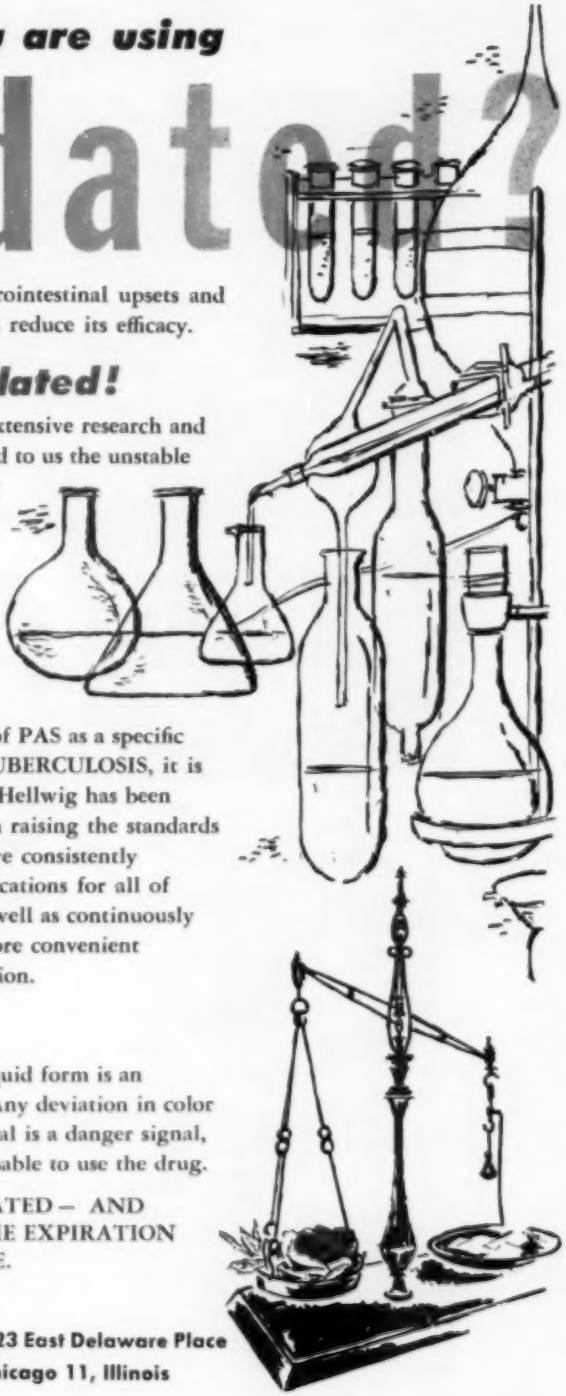

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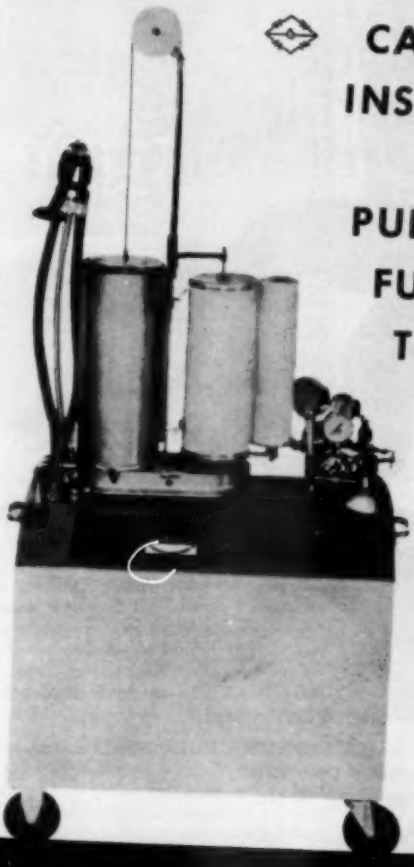

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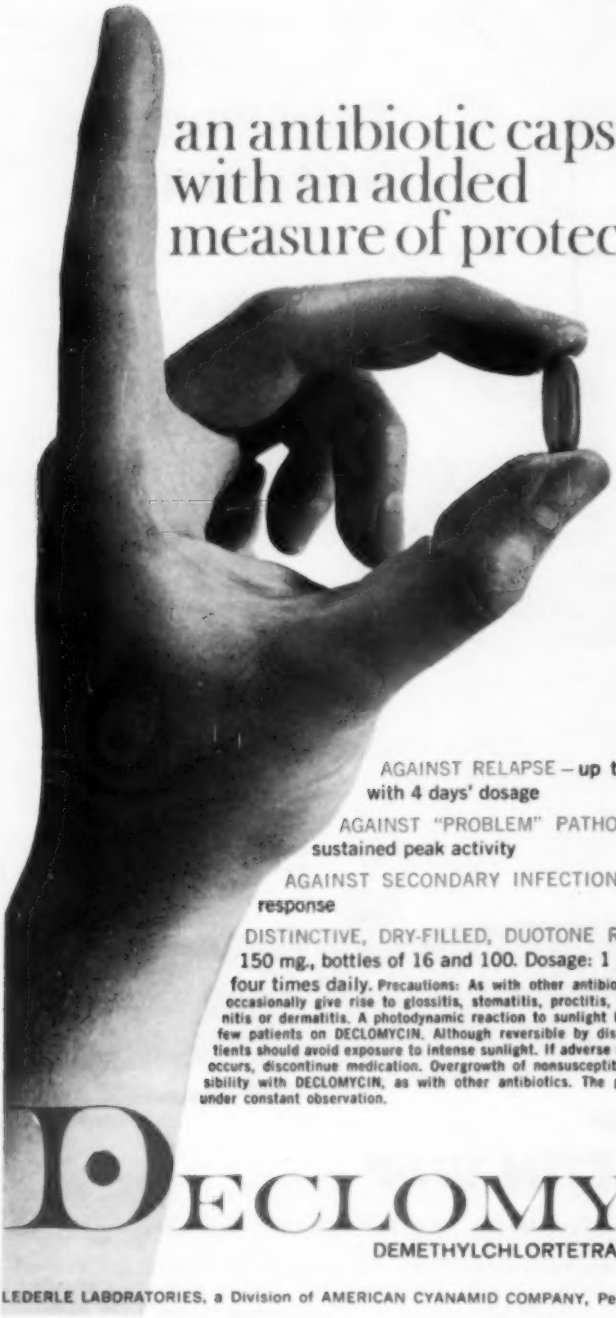

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1. Davey, W. N.: GP 19:107, No. 1, 1959.

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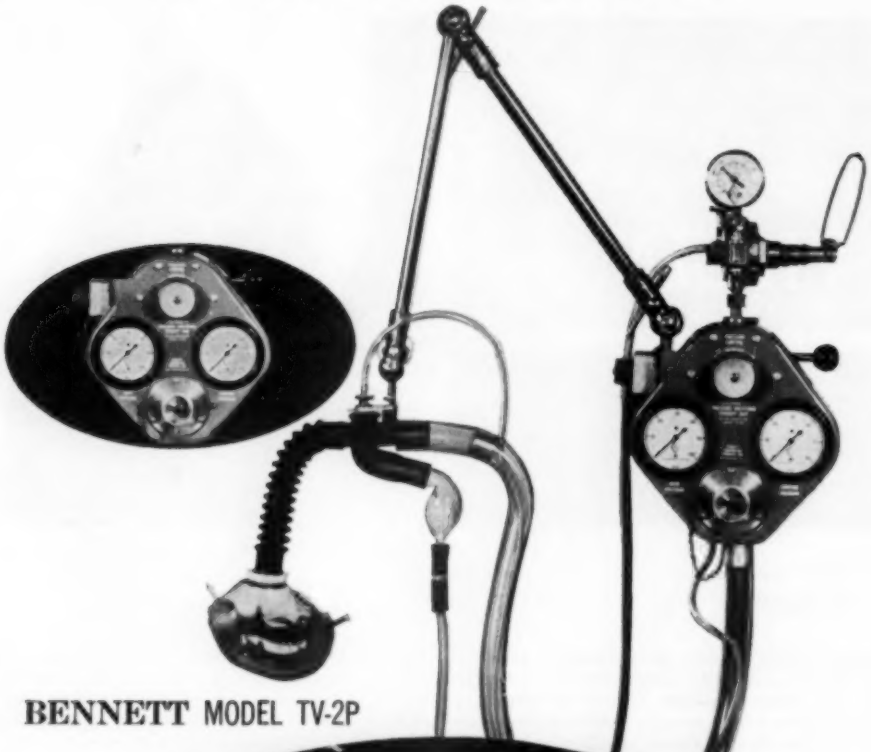

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THE AMERICAN REVIEW
OF RESPIRATORY DISEASES
Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 83 *January 1961* NUMBER 1

THE SO-CALLED PROBLEM OF UNCLASSIFIED MYCOBACTERIA[1]

A Review

C. XALABARDER

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The re-discovery in the last few years of the unclassified so-called "atypical" mycobacteria has aroused considerable attention among bacteriologists and clinicians. Numerous studies on the subject have appeared which attribute importance to them. These unclassified organisms also are referred to as "anonymous," or chromogenic, because they do not all possess the same characteristics as the human tubercle bacilli. In general, they are nonpathogenic for the guinea pig and exhibit poor susceptibility to antimicrobial drugs. Many of them form pigments, and this property has been used in part as the basis for classification.

The problem has elicited much discussion and mutually contradictory results. The whole question would be no more than a laboratory curiosity were it not for the tendency that exists to place the unclassified acid-fast bacilli in a well-defined group independent of other mycobacteria, and the affirmation that the lesions they produce in man are not tuberculous, and are, therefore, of no epidemiologic significance (1–3).

This approach to the subject seems to the writer to be erroneous and misleading. It stems from the oblivion into which the history of bacteriology has sunk at the present moment, for it is only unfamiliarity with that history which can explain the survival of the erroneous and anachronistic theory of "monomorphism" which was postulated by R. Koch. It is also partly the product of the overoptimistic notion that, thanks to the progress recently made in treatment, tuberculosis has almost disappeared. In fact, however, there are still a great many patients with disease entities caused by unclassified mycobacteria whose lesions present the same characteristics as those produced by tuberculosis. Because these organisms do not exhibit a good response to drug therapy, they are arbitrarily given a different name despite their substantial identity. The suggestion also that such disease entities be called "mycobacterioses" would appear deceptive.

To add to the confusion, modern investigators are not in agreement on the terminology employed in the descriptions of the bacilli. They seem to agree tacitly to regard as unclassified those acid-fast bacilli isolated from human specimens but whose properties are similar to those of saprophytic mycobacteria or closely related organisms. Such a group of bacilli, pathogenic in man but not in the guinea pig, has already been described by many workers. In a review of the literature up to the end of 1950, which is not claimed to be exhaustive, the writer has noted that sixty-six investigators have reported the isolation of one or more of such organisms. Unaware of this extensive literature, it has been stated recently (4, 5) that unclassified mycobacteria are rare in Europe despite the fact that a great number of strains were studied by Jensen and Frimodt-Moeller (6) and by Lester (7).

In view of the existing confusion, it appeared worth while to review what was known previously about this whole question which some investigators seem to regard as a recent and highly complex and obscure problem. Its history indicates that, far from being a problem of recent origin, it is not even a problem, since a perfectly satisfactory explanation of the facts exists, thanks to the knowledge already acquired

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on the biology of mycobacteria. In view of the large volume of literature, the writer proposes to confine himself to the earliest or most outstanding investigations into each aspect of the subject, which he believes contribute best to the clarification of the problem of unclassified mycobacteria.

It is well known that scarcely had the echoes of the report of Koch's discovery of the tubercle bacillus died away than other mycobacteria were discovered that were unclassified in relation to the Koch bacillus, whether by reason of their peculiar morphology or because they were nonpathogenic when tested in laboratory animals (8, 9). Even the human tubercle bacillus exhibited various degrees of virulence, giving rise to a new type of disease with no tubercles (10). Three years later, Straus and Gamaleya (11) drew attention to the chromogenic nature of certain mycobacteria and pointed out that it was possible, by changing the culture medium, to alter the appearance of the colonies and to modify the pathogenic potential of the organisms. It is interesting that even then they were called "atypical" acid-fast bacilli, although the name of "paratubercle bacilli" suggested by Marmorek (12) has prevailed since then. From that time on the number of mycobacteria that do not fit into the patterns fixed by Koch has become so large that today it is quite impossible to defend the theory of monomorphism.

According to Koch, the tubercle bacillus invariably presents the following characteristics: (*1*) Development is slow (four to eight weeks) and takes place only in special media; (*2*) the colonies are dry, squamous, friable, and whitish to creamy in color; (*3*) it cannot tolerate changes in temperature; and (*4*) it is invariably pathogenic and strictly parasitic in character. More recently, other properties that appear to be more common in the pathogenic strains have been added, such as the formation of "serpentine cords," niacin synthesis, specific cytochemical reactions, et cetera. It is important to point out that the great majority of characteristics of the pathogenic mycobacteria are in reality biologic deficiencies. This is indicated in table 1 prepared from the data derived from various investigators.

TABLE 1

METABOLIC ACTIVITIES OF MYCOBACTERIA

	Saprophytic Mycobacteria	Pathogenic Mycobacteria	Author
Nitrogen source			
Creatin	+	−	
Urea	+	−	Long, 1922
Air	+	−	Braun, 1924
Carbon source			
Glucose	+	±	Merrill, 1931
Pyruvate	+	−	
Lactate	+	−	
Succinate	+	−	
Malate	+	−	
Citrate	+	−	
Formate	+	−	Lwoff, 1944
Acetate	+	−	
Propionate	+	−	
Oxalate	+	−	
Ethanol	+	−	Long, 1922
Fructose	+	−	
Mannitol	+	−	
Arabinose	+	−	
Galactose	+	−	
Paraffin	+	−	Rahns
Petroleum	+	−	Sohngen, 1913
Metabolizes cholesterol	+	−	Tak, 1942
Oxidizes lactic acid	++	+	Geronimus and Birkeland, 1951
Reduces methylene blue	+	−	Bloch, 1950
Reduces dichlorophenol	+	−	Wilson, 1952
Growth in presence of copper and silver	+	−	Eichbaum, 1933
INHIBITORY ACTION UPON MYCOBACTERIA			
d-l-Serine	−	+	Dubos, 1949
PAS	−	+	Penso, 1951
Thiosemicarbazone	−	+	Lutz, 1950
Isoniazid	−	+	Wacksmann, 1953
Acetylsalicylic acid	−	+	Xalabarder, 1951
Spermine	−	+	Hirsch and Dubos, 1952
Diphtheria anatoxin	−	+	Schain, 1944
Chlorpromazine	−	+	Geiger, 1954

The following properties are generally accepted among bacteriologists as those which characterize the mycobacteria: a bacillary form; acid-fastness; absence of flagella, and motility; reproduction by simple binary fission; and absence of spore formation. Judged by such criteria, however, all mycobacteria are unclassified, since these properties are not constant and vary continually.

There is only one thing about them that is absolutely typical, and that is their great plasticity and adaptability to an extreme variety of environments. To suit those conditions, these organisms will change not only their metabolic patterns and morphologic characteristics, but even their mode of reproduction (13–15). This versatility of mycobacteria is shown in the great number of exceptions, of which all the above-mentioned characteristics are examples, even in the case of pathogenic bacilli. Numerous investigators have pointed them out, but the writer would like to indicate his own observations based on the material of the Barcelona Antituberculosis Institute (table 2).

TABLE 2

THIRTY-FOUR STRAINS OF MYCOBACTERIA WELL-DEVELOPED IN THE SAME CULTURE MEDIUM, WHEN TESTED WITH IDENTICAL METHODS, GIVING THE FOLLOWING RESULTS

	17 Human Strains	17 Saprophytic Strains
	per cent	*per cent*
Cord-forming	76.5	23.5
Chromogenic	29.0	82.2
Niacin-positive	47.0	35.2
Neutral-red binding	94.1	41.2
Nile-blue binding	71.0	35.2
Indophenol-reducing	29.0	64.6

With regard to the slow growth of pathogenic bacilli, to which Moeller (16) attributed so much importance for the purpose of differential diagnosis, it cannot be said that it is a characteristic property. It must be remembered that one of the chief advantages of any new culture medium is based on the fact that mycobacteria develop more rapidly on it than on other media. Some virulent strains can be cultured in ordinary bacteriologic media, while certain saprophytes grow slowly and with difficulty in egg or other special medium. The limiting temperatures are more restricted in the case of virulent strains, as Schlosberger and Pfannenstiel (17) have pointed out, but there are also very exacting strains among the saprophytes and unclassified mycobacteria.

Even the properties attributed to mycobacteria in the bacteriologic handbooks cannot be said to be constant. Acid-fastness is a property which appears only when these organisms have reached a certain stage in their development (18, 19), and they may lose it under certain environmental conditions (20, 21) and even by simple mechanical manipulations (22, 23). Conversely, some bacteria, other than mycobacteria, can acquire acid-fastness when mixed with fats (24, 25).

When mycobacteria different from human tubercle bacilli began to be recognized, it was observed that one of their properties was pigmentation of the colonies, in contrast to the cream color of pathogenic strains. Although there are a great many exceptions to this rule, the presence or absence of pigment has been one of the factors which has been used by Penso and associates (26)—and more recently by Runyon (27)—to classify mycobacteria. And yet, if the past history of bacteriology is studied, it must be noted what little value can be attributed to pigmentation as a reliable criterion for differentiation.

It is easy to divide mycobacteria into colorless and chromogenic strains when the pigmentation of the latter is intense. In such instances many workers would agree that such a criterion might be useful in classification. But the usual result is that pigmentation covers all shades of color and, as slight differences cannot easily be discerned macroscopically or even by a photoelectric cell, each observer makes his own judgment as to whether the Mycobacterium in question is chromogenic or not. This means that any classification based on such a property is only valid for the particular investigator who has made it and is erroneous for others. Thus, for example, Crow and associates (1) isolated 69 unclassified mycobacteria which they designated as yellowish or pale orange but, when these were sent to Runyon, he classified 64 of them as nonchromogenic, which demonstrates that it is really difficult to appreciate slight differences of pigmentation and that the personal element is very real.

In such cases it helps considerably to examine the cultures under Wood's black light; all mycobacterial pigments assume various bright colors which are easy to distinguish. In a study published in 1955 (28), the present writer called attention to the fact that absence of fluorescence under these conditions was typical of virulent strains, but the observation now has to be qualified to some extent. This is true, as a general rule, since most pathogenic strains are not chromogenic and do not fluoresce, but some

virulent strains also contain pigment and, therefore, exhibit primary fluorescence.

In addition to the subjective element of error to which reference has been made above, it also has been known for a very long time that the pigmentation of mycobacteria is not a constant or permanent property of all chromogenic strains. Some organisms, even pathogenic, appear colorless in one culture medium and pigmented in another (29–34). Small variations in composition of the same medium also have an influence on pigmentation, which is enhanced by the addition of iron (35), glycerin (34), bovine serum (36), or glucose (37). Sometimes significant differences in the chromogenic character of the culture can be observed, depending on its age, generally increasing in color intensity the older it is (38–40). An increase of pigmentation also is produced by changes in pH (41), by roentgen rays (42), exposure of cultures to white light (43, 44), or the photons of different wave lengths (45). Sometimes the chromogenic property of mycobacteria will appear and disappear spontaneously and unpredictably (46) or through dissociation (43, 47–49).

The presence of oxygen is critical for pigment formation. In hermetically sealed tubes, pigmentation does not take place even after several weeks of incubation (50) but, if air is allowed to enter the cultures, especially with simultaneous ultraviolet irradiation, pigmentation takes place rapidly through oxidation of a precursor which is invariably synthesized by chromogenic mycobacteria (45).

For all of these reasons, it is obvious that pigmentation cannot adequately serve as a basis for the classification of mycobacteria, since it is inconstant and varies according to circumstances (51). On the other hand, the presence of pigment is not an exclusive property of unclassified and paratubercle bacilli. Some pathogenic mycobacteria are faintly pigmented, and paratubercle bacilli can be more harmful than is generally thought.

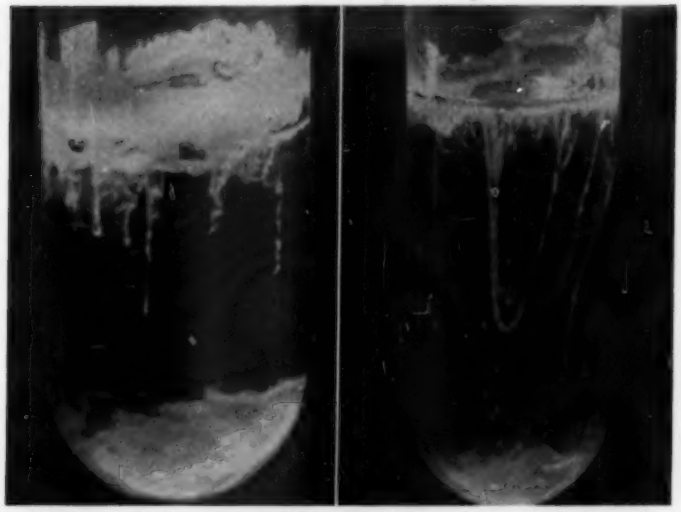
Moreover, the formation of "serpentine cords" is not a constant property of virulent mycobacteria. It is true that there is a remarkable correlation between this characteristic and pathogenicity, but many exceptions have been demonstrated to exist among pathogens, depending on a variety of environmental factors. Some nonpathogenic strains also may exhibit various degrees of cord formation (52–60). In the Institute several saprophytic strains have been observed which exhibit cording, visible in some instances macroscopically (figures 1 and 2).

Cytochemical reactions (61–63) also are of limited value as virulence tests. Hauduroy (64) has published an excellent review on this subject, and in this laboratory discrepancies have been found in the results of 50 per cent of the various strains tested. It should also be pointed out that the same strain may exhibit either positive or negative cytochemical reactions depending upon the age of the culture (65, 66).

The remarkable versatility of the mycobacteria is not a matter of recent observation. Considerable variations in their morphology have been noted since the time of Metschnikoff (67) who drew particular attention to the no less pathogenic dwarf forms. His observations were confirmed by Strauss (38) who was able to witness the recovery of these organisms to their original size after reinoculation into glycerol medium. Nocard and Roux (9), Ferran (20), and many other investigators described unclassified forms. The reluctance of some workers to admit the great capacity for variation possessed by the mycobacteria does not appear tenable in view of the numerous reports concerning the wide variety of forms which these organisms are able to assume under various conditions. This is sufficient proof of their plasticity.

Koch's monomorphist theory was firmly held for so many years that a large amount of work was devoted to the search for a reliable means of differentiating between the human and bovine tubercle bacilli as well as other types of mycobacteria. It was believed that, if this were achieved, it would afford an invaluable weapon in the fight against tuberculosis. It was also recognized that the objective characteristics which were thought to be constant for each type of organism were in fact only temporarily so, since it was possible by means of various laboratory procedures, or their passage through certain animals, to effect a transformation of one variety into another (68–72). A good summary of the whole problem will be found in the excellent book by Darzins (73).

What gave the "coup de grace" to the theory of monomorphism was the discovery, first mentioned by Baerthlein and Tojoda (74) and later confirmed by many bacteriologists, of the



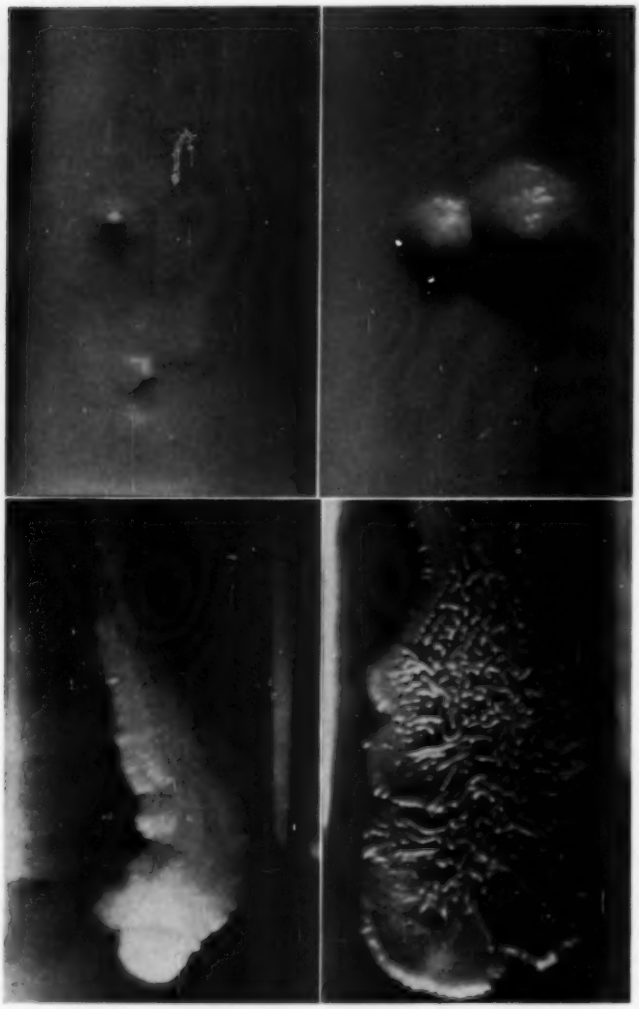
FIGS. 1 and 2. The saprophytic strains *A* and *C*, isolated from a watertap, cultured in glycerin broth clearly show microscopic and macroscopic cords.

spontaneous dissociation of *M. tuberculosis* into rough, smooth, and chromogenic variants. Birkhaug (43) proved that the morphology of rough and smooth colonies bears no strict relationship to the virulence of the organisms, but that the chromogenic variants have always exhibited little or no pathogenicity for guinea pigs. He was able to obtain artificially all of the variants produced by dissociation from a single bacillus which was isolated by micromanipulation. This is the clearest possible proof of the plasticity of mycobacteria. In his excellent monograph, Birkhaug states that pigmentation varies according to whether these bacteria are or are not exposed to the light, a property to which, perhaps, too much importance is attached nowadays.

The earliest investigators were concerned from the beginning with the very problems which some workers today are raising in their own minds; namely, is it possible for nonpathogenic mycobacteria to acquire virulence; is it possible also for pathogenic strains to lose their virulence in the course of time and eventually become harmless saprophytes. In other words, do the pathogenic strains form a well-defined taxonomic group, distinguished from the saprophytic strains by clear-cut borderlines across which no interchange is possible?

The impossibility of such interchange was obstinately defended by Koch despite the larger number of investigations proving not only the morphologic but also the pathogenic mutability of the mycobacteria. Ferran (20), Arloing (21), and Auclair (75) were able to cause various pathogenic mycobacteria to lose their virulence by passage in liquid medium. Since 1891 it has been known that tubercle bacilli can be made less virulent by serial inoculation in the same medium after long periods of time (11). The presence of fatty acids (76, 77) or the action of heat (78, 79) may lessen the virulence and retard the development of mycobacteria. If tuberculous tissues are incubated at 37°C. from two to eight weeks, tubercle bacilli are so weakened that they are no longer pathogenic for guinea pigs (80). Tubercle bacilli which have been in contact with gastric juice produce only local lesions which can recover spontaneously (38).

More recently, it has been demonstrated that the virulence of these organisms diminishes when the culture medium is poor in phosphate content (81) or when the bacillary deoxyribonucleic acid is modified by chemical means (82). It also has been shown that tubercle bacilli become nonpathogenic for guinea pigs after passage through reptiles or frogs (83). On the other hand, the enhanced virulence of certain strains after repeated passage through susceptible animals



FIGS. 3 and 4 (Top). These strains, isolated from human patients who have had long courses of antimicrobial drugs, show unclassified colonies, are chromogenic, rapid growing, and niacin negative. Notwithstanding, they are pathogenic for guinea pigs.

FIGS. 5 and 6 (Bottom). Saprophytic strain *B*. Striking gross morphologic differences attained by changing its source of *C*. Figure 5 (left)—cultured on glycerin agar; figure 6 (right)—cultured on mannite agar.

was demonstrated (84–86). The recrudescence of virulence also occurs spontaneously from time to time (87).

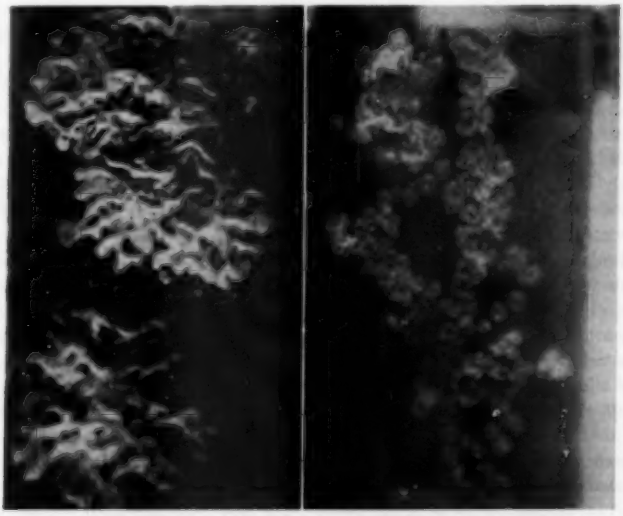
It is not only virulence that varies. The morphology of mycobacteria also undergoes marked changes when they are subjected to external physical influences such as pressure, shaking, various kinds of radiation, et cetera, or to chemical influences. The literature is full of examples which could be cited in evidence, but the electron microscope has furnished the final proof (88–91). The present writer has recorded

the different morphologic forms assumed by the same strains of mycobacteria cultured the same number of days, but in different media (92). This is in complete accord with the gross changes observed in the colonies (figures 3–8).

The adaptability of mycobacteria to their environment is exercised by them in no less degree when the changes are brought about under the influence of antimicrobial drugs. Under such conditions the organisms assume unusual forms, yet they retain enough vitality and regenerative power to resume their normal morphology when the environment is once more favorable to them (93). It is a remarkable fact that, if antimicrobial drugs are added to cultures of tubercle bacilli in sublethal doses but continuously, the drugs are able to modify these organisms to such an extent that they acquire all of the morphologic and biologic characteristics, including pigmentation, of atypical or attenuated strains (94–96).

The diminished pathogenic capacity observed in strains which have acquired resistance to antimicrobial drugs is not a phenomenon confined to the mycobacteria, but also has been found to occur among other bacterial species (97, 98). These observations could well explain why antimicrobials are ineffective in unclassified mycobacteria since they have already developed by adapting themselves to the changes of the environment resulting from the presence of the antimicrobials (92).

The relationship that exists between unclassified mycobacteria and virulent tubercle bacilli is something that early investigators had no difficulty in observing. On the other hand, the possibility of a transformation of saprophytic mycobacteria or attenuated tubercle bacilli into pathogenic mycobacteria, or vice versa, was the subject of great controversy at the beginning of this century. The prevailing idea was that such a reversible transformation was impossible. This was the conclusion arrived at by Calmette at the IV International Conference at Lausanne in 1924. His great influence enabled this concept to prevail then, and it has continued to the present. However, many investigators at this Congress asserted that there is a close relationship between these two groups of organisms, and among other things it was demonstrated that they have common antigens (99, 100). Cross reactions with tuberculin from paratubercle and tubercle bacilli had been known since 1905, and the phenomenon was later confirmed by many investigators (101–106). With modern techniques, using double precipitation on agar-gel and immuno-electro-



FIGS. 7 and 8. In this human strain, the differences become much more evident when cultured in different media. Figure 7 (left)—cultured on glycerin agar; figure 8 (right)—cultured on ascites fluid agar.

phoresis, it also has been demonstrated that all mycobacteria have one or more common antigens (107).

These observations strengthen the evidence for a common origin of all mycobacteria, a concept accepted by many investigators long ago (108–116), but all doubt appears to have been removed since it has been possible to transmit the pathogenic character artificially to saprophytic strains by culturing them in the presence of filtrates from other virulent strains, even when the latter had been killed by heat (117). Later, it was demonstrated that the transforming factor is a nucleoprotein constituted by deoxyribonucleic acid and another component similar to histones. Either of the two components apart modifies saprophytic strains, but the modification is not permanent. To obtain the full effect of virulent transformation, reconstitution or synthesis of the components is necessary (118).

Having observed later that it is possible to modify virulent deoxyribonucleic acid chemically, with consequent substantial change in its physicochemical and biologic properties, virulent mycobacteria were cultured in the presence of these chemical substances that modify deoxyribonucleic acid. In addition, it was possible to induce in certain virulent organisms all of the characteristics of the saprophytic mycobacteria, including pigmentation and loss in virulence (118, 119). If, then, it is possible to induce the reversibility of properties *in vitro* between saprophytic and pathogenic organisms, the same thing may occur *in vivo* as a consequence of innumerable biochemical factors working in the host, particularly if these factors are subject to the influences exerted by drugs. This could well explain the greater frequency of isolation of unclassified mycobacteria which has been observed since the advent of antimicrobial drugs, and the fact that these organisms make their appearance when virulent tubercle bacilli disappear and the patient is on the way to recovery.

It is generally admitted that the chief difference between virulent and saprophytic mycobacteria lies in the fact that the former produce progressive lesions which cause death, while the latter are quite innocuous and cause only local lesions at the site of inoculation. This is a mistaken opinion, since it is based on the somewhat arbitrary practice of killing laboratory animals within too brief a period. In the case of guinea pigs, this has been fixed at approximately eight weeks because most animals inoculated with virulent organisms generally die within this period. Saprophytic strains generally do not produce apparent lesions within two months. When guinea pigs were kept for a longer period, up to many months, however, characteristic histologic lesions were seen. These consisted of epithelioid and giant cells with marked fibrotic reaction similar to that observed in slowly developing human tuberculosis and sometimes simulating exactly the histologic picture characteristic of the collagen diseases (121). When material from these lesions was inoculated into other guinea pigs, it caused caseation of the mediastinal lymph nodes with fibrotic alterations in all organs (figures 9, 10).

It may be recalled that during the last years the lesions produced by virulent tubercle bacilli in the guinea pig were characterized by their predominant granuloma formation with little or no caseous necrosis and with little effect on the general condition of the animals. In many instances the disease process took place without any dermal sensitivity to tuberculin, at least to the standard tuberculin. Today, survival of guinea pigs infected with the same number of organisms used in previous years is very much longer. This spontaneous attenuation of virulence was noted long before the discovery of antimicrobial drugs.

In a comprehensive and objective study on the problem of experimental tuberculosis, Tarshis and Frisch (122) comment on various studies in connection with the possibility that error might arise from the mistaken identification of certain lesions as tuberculous which, in fact, they are not. This possibility does exist, but it should be pointed out that it is very often the opposite that occurs. Some investigators have indicated that the lesions are not tuberculous on the basis that there is no caseation or because there are no detectable acid-fast bacilli. But they overlook the fact that there are tuberculous disease processes which do occur without caseation and, since acid-fastness is by no means a permanent characteristic of mycobacteria, the organisms could be present in the tissues, but are not recognized or found by the methods usually employed to detect them.

Much of the confusion that prevails on this

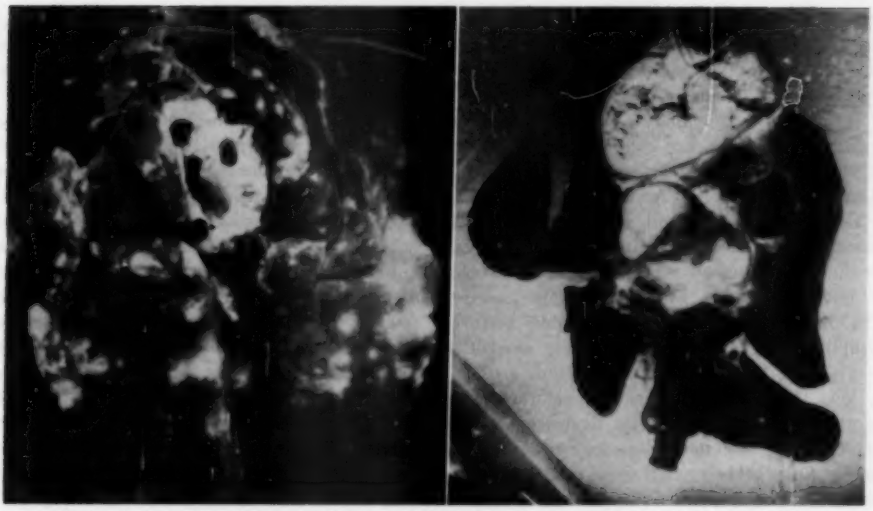


FIG. 9 (Left). Tuberculous lesions caused in a guinea pig by intradermal inoculation of a chromogenic, acid-fast organism isolated from a watertap. The animal was killed after thirteen months' observation without showing external signs of illness. These lesions were reinoculable.

FIG. 10 (Right). The reinoculation of material from the lesions shown in figure 9 into another guinea pig caused an enormous caseating involvement of the mediastinal nodes. This disease also was of long evolution and the chromogenic organism was recovered by culture.

subject is due to the fact that many of the unclassified mycobacteria have been isolated from the gastric contents. This is a routine clinical technique, but its value is highly debatable since, in addition to the sputum swallowed, all of the countless acid-fast bacilli found in food find their way into the stomach and may remain there long enough to be detected when the stomach is washed out. Even pathogenic tubercle bacilli which do not belong to the patient himself may be found there. One of the most virulent strains at the Institute was isolated from the gastric washing of a perfectly healthy child who was kept under observation for twenty-five years without ever developing tuberculosis. More unclassified mycobacteria have, therefore, been found since cultures have been made from the contents of the stomach, especially when sulfuric acid is used instead of sodium hydroxide in processing the specimens (7).

On the other hand, acid-fast bacilli are seldom found in the gastric washings of healthy persons. This has been confirmed again recently by Burrows and Barclay (123) who isolated only one atypical organism out of 399 cultures from the gastric contents of nontuberculous persons. They conclude: "These organisms are infrequent incidental saprophytes in the population as a whole. The frequent isolation of such organisms in tuberculosis sanatoriums would seem related in some way to tuberculous infection or to the chemotherapy thereof." Tarshis (124) has made similar observations.

The experimental data indicate that there is no justification to classify mycobacteria into mutually independent groups. If the organisms in each of these groups were the cause of different diseases requiring different specific treatment, this classification would be reasonable. But it has been indicated that all mycobacteria are capable of producing tuberculosis, or a disease process closely resembling this disease, in susceptible animals. The differences observed between various strains of mycobacteria are simply differences in pathogenic response in a given host, which may be quite different in other species or in different individuals of the same species. For this reason, well known since the beginning of the century but too often underestimated, the belief that an organism which does not kill a guinea pig in a period arbitrarily fixed at approximately eight weeks has no clinical or

epidemiologic significance is unjustifiable. Neither Hansen's nor Eberth's bacilli produce disease in the guinea pig, yet no one would deny that leprosy and typhoid fever are serious diseases. It is also wrong to classify as nonpathogenic an organism which does not kill a guinea pig. Under other conditions, it may well be virulent for other animals as well as for man.

Moreover, there is no justification for the assertion that the disease (fatal in some cases) produced in man by unclassified mycobacteria is not tuberculosis but some other disease. The histologic and roentgenographic picture, symptomatology, and course of this disease is very similar to that of slowly developing tuberculosis. The limited response to chemotherapy is another point of similarity for, as every clinician knows, it is a common experience nowadays that tuberculosis is becoming more and more resistant to drugs, which do indeed attenuate the disease from the clinical point of view, but leave the problem of eradicating the tuberculous infection as much a chimera as it was before. In a recent pertinent report, McDermott (125) has commented significantly on this old problem of dormant infections caused by bacteriostatic drugs, which is assuming greater importance in human pathology.

Another statement sometimes made is that unclassified mycobacteria are evidently not contagious in view of the dearth of positive tuberculin test results obtained on contacts. This argument is not decisive since all clinicians know that there is an ever-increasing number of tuberculous patients who are Mantoux negative (126) and that experimental tuberculosis not infrequently runs its course in guinea pigs with only transient or negative tuberculin skin reactions, as pointed out above.

This spontaneous change in the biology of tuberculous disease is also shown in the pathologic response of infected laboratory animals. Formerly, the lesions produced by virulent strains of tubercle bacilli could be easily distinguished from those caused by attenuated strains. With the latter there was a predominance of epithelioid and giant cells, but no caseation or peripheral lymphocytic reaction; in other words, the lesions did not contain typical Köster's follicles; whereas now—before the introduction of antimicrobial drugs, certainly—tuberculosis in guinea pigs and in man has gradually acquired similar characteristics. It develops more slowly and becomes more benign anatomically, with fewer and less pronounced external signs of illness, so that it is now difficult or impossible (in some cases) to say with certainty whether a particular tuberculous lesion is produced by a virulent or by an attenuated organism. In the case of laboratory animals, it may be conjectured on the time it would take for disease to develop, but this can hardly be done with any accuracy in man.

Bacteriologists have established their systems of classification on the basis of assigning fixed, precise, and immutable characteristics to each group of organisms. This was necessary for didactic purposes in the purely descriptive stage of bacteriologic research. However, as the agreement was unilateral, the result was that the bacteria, not aware of this convenient human arrangement, have continued their normal biologic processes, which consist in a steady evolution and adaptation to the greatest variety of environmental conditions, so that now there is great discrepancy between the real state of affairs and that envisaged by our well-intentioned blueprints. In order to overcome this difficulty, recourse to two subterfuges appeared necessary: occasional modification of the taxonomic pattern and, in the case of tuberculosis, formation of a separate group containing all of the mycobacteria which do not play the game according to the rules fixed by the committee, dubbing them "atypical" acid-fast bacilli and segregating them into a group apart from other mycobacteria. This procedure, far from clarifying matters, has led to considerable confusion and mistaken conclusions dangerous to the interests of epidemiology. Pergola (127) protested against all of this fifteen years ago in the following statement: "There is no justification for the creation of a type of atypical bacilli, since their lack of pathogenic power depends on other factors and not on the bacillus itself."

Summary

The studies which have been reviewed seem to prove that mycobacteria constitute a single group of organisms but with gradations of progressive change. Their final and most clear-cut stage, involving loss of many of their biologic properties but which happens to be toxic for the susceptible animals, is represented by the pathogenic strains. Such a change is reversible and,

therefore, a saprophytic strain may, under certain conditions, turn into a virulent one, and vice versa. We have brought this about *in vitro*, so there is little reason to doubt that it also may take place *in vivo*.

This concept of the phylogenetic unity of all mycobacteria has been defended by a great many investigators since the beginning of the century and has been fully demonstrated by recent research. Therefore, it is not surprising that the so-called "atypical" or "unclassified" mycobacteria are found to originate from pathogenic organisms, whether spontaneously or under the influence of antimicrobial drugs. The contrary opinion (128) is based on theoretic considerations which are quite reasonable but which, for lack of convincing proof, cannot be accepted at the moment.

Sumario

El Llamado Problema de las Micobacterias Inclasificadas. Reseña

Los estudios aquí repasados parecen demostrar que las micobacterias constituyen un solo grupo de microbios, pero con grados de alteración evolutiva. Su etapa final y más bien definida, comprendiendo la pérdida de muchas de sus propiedades biológicas, pero que resulta ser tóxica para los animales susceptibles, está representada por las cepas patógenas. Esa alteración es reversible y, por lo tanto, una cepa saprofita puede, en ciertas condiciones, volverse virulenta, y viceversa. El A. hizo esto *in vitro* y hay pocos motivos para dudar que puede también tener lugar lo mismo *in vivo*.

Este concepto de la unidad filogenética de todas las micobacterias ha sido defendido por una multitud de investigadores desde los comienzos del siglo y ha quedado plenamente demostrado por recientes pesquisas. Por ello, no es sorprendente descubrir que las llamadas micobacterias "atípicas" o "inclasificadas" tienen su origen en microbios patógenos, ya espontáneamente o bajo el influjo de drogas antimicrobianas. La opinión opuesta (128) se basa en consideraciones teóricas que son bastante lógicas, pero que, a falta de pruebas convincentes, no cabe tomar en cuenta por ahora.

Resume

Ce qu'on appelle le problème des mycobactéries non classées. Une revue

Les études qui ont été passées en revue semblent prouver que les mycobactéries constituent un groupe unique d'organismes mais qu'elles possèdent des degrés de changement progressif. Leur stade final et le mieux défini, comprenant la perte de beaucoup de leurs propriétés biologiques, mais qui dévient toxique pour les animaux susceptibles, est représenté par les souches pathogènes. Un tel changement est réversible et, par conséquent, une souche saprophyte peut, dans certaines conditions, se transformer en une souche virulente, et vice versa. Nous sommes arrivés à cela *in vitro*, aussi y-a-t-il peu de raison de douter que cela peut se passer *in vivo*.

Cette conception d'unité phylogénétique de toutes les mycobactéries a été défendue par un grand nombre de chercheurs depuis le début du siècle, et a été complètement démontrée par des recherches récentes. Par conséquent, il n'est pas surprenant que ce qu'on appelle les mycobactéries "atypiques" ou "non classées" soient trouvées proceder des organismes pathogènes, que ce soit spontané ou sous l'influence de médicaments antimicrobiens. L'opinion contraire (128) est basée sur des considérations théoriques qui sont tout à fait raisonables mais qui, par manque de preuve convaincante, ne peuvent pas être considérées pour le moment.

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DILATED BRONCHIAL MUCOUS GLANDS IN CHRONIC BRONCHITIS, A NEGLECTED MORPHOLOGIC FINDING[1,2]

Correlation of Bronchoscopic and Bronchographic Appearance

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Introduction

Although it has been well over twelve decades since hypertrophy of the bronchial glands following chronic bronchitis was described by Rokitansky (1), and glandular cysts found in the posterior tracheal wall were reported just ninety years ago by Gruber (2), only in recent years has it been possible to demonstrate such findings in the living patient. The first reports of roentgenographic (bronchographic) visualization of hypertrophied, dilated bronchial glands were published in the foreign literature (3–10, 13). Reports on bronchoscopic visualization of the openings to these dilated mucous glands cannot be found in the standard text books on bronchoscopy and peroral endoscopy. The first and only reference to endoscopically seen dilated bronchial gland ducts is made by Duprez and Mampuys (3), who approached this pathognomonic finding through six investigative methods. Four of them were post mortem and there were two approaches *in vivo*, namely, by bronchography and bronchoscopy, utilizing the magnification afforded by the bronchoscopic telescope. There have been no reports or descriptions of such findings in the domestic literature on bronchoscopy. Since these findings are rarely demonstrable on direct vision, the telescopic magnification (4×) is most essential in visualizing the finer details of the bronchial mucosa.

The writer has used the right-angle telescope routinely in all bronchoscopies since 1949 and the forward-oblique telescope in suitable cases, that is, when the paucity of secretions allowed for adequate visualization. The frequently seen details presented themselves as "areas of pitting," "puckering of the mucosa," "shallow or blind indentations," and small "holes." They were thus noted and were estimated to be from less than 1 mm. in diameter to slightly more than 2 mm. in diameter, taking the telescopic magnification into consideration. Initially the small areas of pitting, whether solitary or multiple, were considered to be anomalies of the bronchial mucosal pattern. When the issue of predominance of bronchogenic spread of pulmonary tuberculosis by caseous lymph node perforation was raised by Schwartz (11), it was believed that detection of such areas of perforation should be demonstrable endoscopically. Therefore, beginning in 1952, the present writer adopted the policy of noting in detail any finding of scarring, pitting, or indentation in the mucosa, and this procedure has been followed in every single bronchoscopy in which such findings were presented telescopically. For a number of years it was even suggested in these bronchoscopic reports that the areas of pitting, reactionless as they were, represented residues or sinus tracts from previously perforated lymph nodes.

Material and Methods

In order to arrive at a figure representing the rate of occurrence of this pathognomonic morphologic sign of chronic bronchitis, a study was undertaken of two series of bronchoscopies. One series consisted of all of the bronchoscopies performed at the Mount Wilson State Tuberculosis Hospital, Mount Wilson, Maryland, from 1949 to mid-1956; the other comprised 250 bronchoscopies performed at a general hospital, Church Home and Hospital, Baltimore, Maryland, from January, 1957 to May, 1960, inclusively (tables 1, 2, and 3). In the first series, all bronchoscopic operative notes which are now part of the patients' records were reviewed. No confirmatory bronchograms were available, since bronchographic study was made in only a few cases. Apart from purely diagnostic bronchoscopies in the nontuberculous cases (8 per cent), most bronchoscopies were performed routinely prior to contemplated chest surgery for tuberculosis, or as follow-up studies in postoperative cases showing endobronchial complications such as suture granulomas, infected bronchial stumps, or tuberculous reactivation elsewhere. This indicates that many more

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TABLE 1

"Positive" Findings of Dilated Bronchial Glands ("Sinus Tracts," "Pitting," "Minute Holes" and "Blind Recesses") Seen Telescopically in the Series of Bronchoscopies Performed at Mt. Wilson State Hospital, Mt. Wilson, Maryland, from 1949 to mid-1956

Number of Bronchoscopies	Etiology (Diagnosis)		Number of Bronchoscopies Showing Gland Ducts Telescopically	Number of Patients Bronchoscoped	Number of Patients with "Positive" Findings
	Tuberculosis	Nontuberculous Disease			
896	820	76	94	654	72
Per cent of total	92	8	10	Per cent of patients	11

TABLE 2

Demonstration of "Positive" Findings of Dilated Bronchial Gland Ducts by Bronchoscopy, Bronchography Alone, as well as by Both in a Series of 250 Bronchoscopies Performed at a General Hospital (Church Home and Hospital, Baltimore, Maryland) from 1957 through mid-1960

Number of Bronchoscopies	Etiology Based on Discharge Diagnosis		Number of Bronchoscopies Showing Telescopic Findings	Number of Bronchograms Demonstrating Findings Without Prior Endoscopic Visualization	Number of Demonstrations of Dilated Bronchial Glands by Bronchoscopy and Subsequent Bronchography	Total Number of Demonstrations of Dilated Bronchial Glands
	Neoplasm	Inflammatory				
250	71	179	72	5	33	77
Per cent	28	72	29	2	13	31

bronchoscopies were performed (896, table 1) than the number of patients subjected to this procedure (654, table 1). The series expresses rather conservative figures because, between 1949 and 1952, the finding of pitted areas, holes, or sinus tracts was not always noted unless an abundance was presented. Since mid-1952, it is certain that any such finding was noted in the operative report.

It should be emphasized that any sinus tracts seen in bronchial stumps or stump granulomas after resection were not included as the finding of bronchial gland ducts or sinuses, since their etiology obviously was connected with the preceding surgery and a previously present foreign body (silk suture material). The finding of pitting, puckering, or sinus tracts in the mucosa, which are now known to be the openings of dilated bronchial glands, was noted in 94 bronchoscopies or 10 per cent of the bronchoscopies performed (table 1). Of 654 patients investigated, 72 showed this finding on bronchoscopy by telescopic examination. Only one case was believed to have openings large enough and with appropriate drainage and reaction of the mucosa to fall into the category of perforation of a caseous tuberculous lymph node.

It is worth noting that a much larger series of bronchoscopies in tuberculosis institutions is available at the Victor Cullen State Hospital, Henryton Sanatorium, and the Tuberculosis Division of Baltimore City Hospitals, comprising at least 2,000 more bronchoscopies over a period of fourteen years. However, the copies of the operative reports are attached to their respective charts, and it would be necessary to ascertain whether they are still stored. This would be a most tedious task and might not prove worth while since not all of these bronchoscopies had the advantage of telescopic aid.

The second series was compiled from the bronchoscopies performed in one single general hospital (table 2) and comprised 250 bronchoscopies. No breakdown into the number of patients was rendered here, since re-bronchoscopies were relatively few; practically all cases presented diagnostic problems, and the procedure was carried out in the process of pulmonary study. An attempt was made to classify these bronchoscopies on the basis of confirmed neoplasia versus inflammatory change. Bronchoscopies were performed in 71 neoplastic conditions, as compared with 179 non-neoplastic, inflammatory, or degenerative conditions. This does not preclude, of course, the existence of any antecedent inflammatory pathology such as acute or chronic bronchitis,

TABLE 3

PREDOMINANT PATHOLOGY PRESENT IN 77 CASES WHICH PERMITTED DEMONSTRATION OF "DILATED BRONCHIAL GLANDS," "SINUS TRACTS," "DIVERTICULA," "PITTING," AND "HOLES"

Number of Cases	Cancer of Bronchus	Undetermined Neoplasm	Pulmonary Tuberculosis	Bronchitis	Asthma; Allergic Bronchitis	Emphysema; Obstruction with Bronchitis	Bronchiectasis; Chronic Lung Abscess	Unresolved Pneumonia	Unexplained Hemoptysis	Pleuritis; Empyema	Lung Cyst
77	11	1	7	9	4	21	15	3	3	2	1
Per cent of	14	1	9	12	5	27	20	4	4	3	1

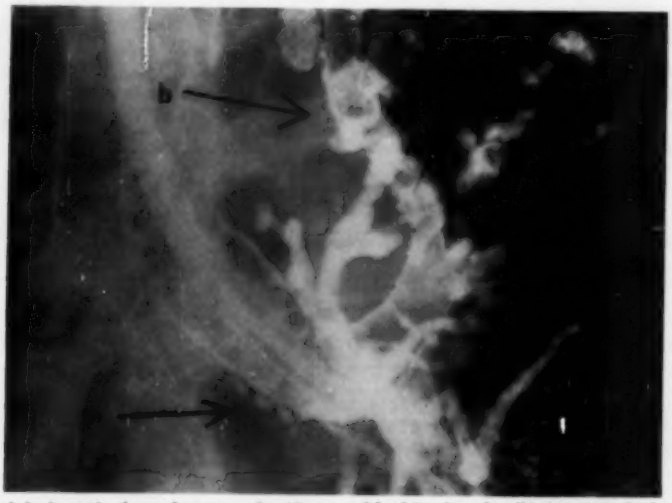

FIG. 1. Partial view of a bronchogram of a 42-year-old white female. (*b*) Arrow pointing to extensive bronchiectasis of left upper lobe. (*m*) Dye-filled, dilated bronchial glands of left main bronchus.

emphysema, or healed pulmonary tuberculosis. The telescopic visualization of openings of dilated bronchial glands was found in 72 bronchoscopies (table 2) or 29 per cent of all bronchoscopies performed. There were 5 bronchoscopies (2 per cent) in which there was no mention of any such finding in the operative report and in which a clear-cut demonstration of dilated bronchial glands was observed in the subsequent bronchogram.

In a review of the operative reports of these 5 cases, *Case 1* (figure 1) showed "increased redness of a fine velvety character, beginning 1½ cm. peripheral to the carina, on the medial and posterior walls of the left main stem bronchus, with the mucosa assuming a granular character and showing bloody oozing upon slightest instrumentation." The other 4 cases offered technical difficulties such as definite tracheobronchial distortion, spasm, and extreme dyspnea, combined with an attitude of panic on the part of the patient. Therefore, perhaps the effort at adequate visualization was insufficint to establish the finding. A total of 33 bronchoscopies showing endoscopic evidence of bronchial gland openings was followed by a confirmatory bronchographic demonstration of extraluminal contrast-filled small sacs that were identical to those labeled as dilated bronchial glands in the literature. In no instance did the bronchography precede the bronchoscopy, since it is the writer's policy and that of his referring colleagues to obtain endoscopic mucosal inspection as well as regional aspiration of secretions and cell washings before the introduction of bronchographic contrast medium (oily Dionosil®). The opposite procedure could cause possible alteration of cells as well as secretions.

CASE REPORTS

A few case reports are presented with accompanying illustrations:

Case 1. A.P., age 42, a female, with the diagnosis of bronchiectasis was mentioned before (figure 1).

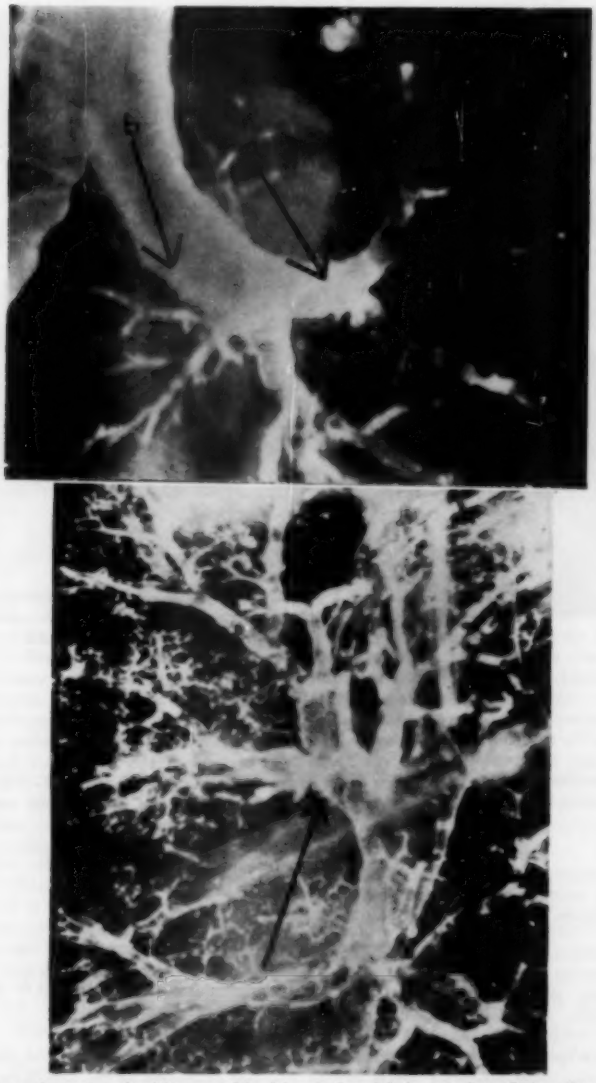

FIG. 2. (Top) Bronchogram of a 53-year-old white male with incomplete filling due to severe bullous emphysema. Oblique view of left bronchus. (*a*) Arrow pointing to dye-filled, dilated bronchial glands in floor of left upper lobe bronchus. (*b*) Arrow pointing to medial wall of left main stem bronchus, showing multiple small indentations, caused by dilation of bronchial glands.

FIG. 3. (Bottom) Upper two-thirds of bronchogram of right side of a 49-year-old white female. Arrow points to multiple, dye-filled bronchial glands in floor of right upper lobe bronchus. Some peripheral beading and distortion of bronchi can be noted also.

Case 2. R. W., age 53, was a male with the diagnosis of bullous emphysema. There was endoscopic evidence (August 18, 1959) of puckering and scarring of the margin and the inferior and posterior portions of the left upper lobe bronchus by right-angle telescopic inspection; there were also crevices and irregular indentations within the atrophic mucosa between the left upper lobe divi-

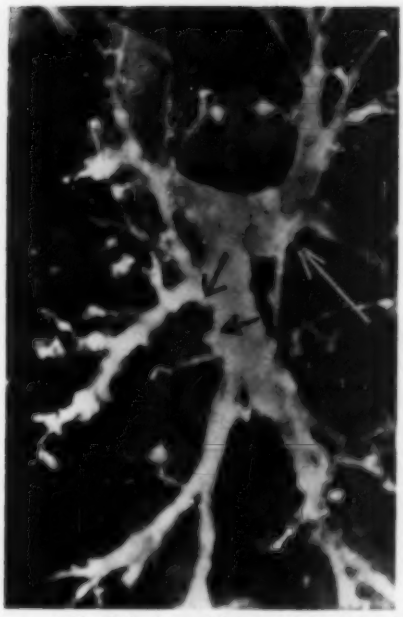

FIG. 4. Partial view of bronchogram of a 34-year-old white male, on left. White arrow points to floor of left upper lobe bronchus where small indentations are caused by dilated bronchial glands. Black arrows point to larger diverticula-like dilatations in the intermediary left lower lobe bronchus. "Beading" and peripheral "pools" and "spiders" are also seen, indicating a high degree of chronic deforming bronchitis and questionable bronchiolectasis.

sion and the superior segment branch of the left lower lobe. The bronchographic demonstration may be seen in figure 2.

Case 3. G. C., age 49, was a female, whose diagnosis was arrested fibroid tuberculosis and chronic allergic bronchitis. She was bronchoscoped on September 8, 1959. Telescopic visualization of the right upper lobe orifice showed numerous pitted holes somewhat larger than usual, located between the inferior and posterior walls of the right upper lobe bronchus before division into the anterior and posterior branches. Several holes were also seen within the right main stem bronchus adjacent to the inferior margin of the right upper lobe orifice. A total of ten openings was counted endoscopically. Subsequent bronchography (September 10, 1959), illustrated in figure 3, clearly demonstrated these contrast-filled structures imitating "diverticula," although far less than ten were seen in the bronchogram.

Case 4. E. S., a 34-year-old male, was diagnosed as having obstructive emphysema and bronchiolectasis. He was first bronchoscoped on April 16, 1957, at which time telescopic inspection of the inferior wall of the left upper lobe orifice showed several puckered areas, two of which were distinct openings, estimated to be less than 1 mm. in diameter. The findings were interpreted then as openings of dilated bronchial glands, but no bronchography was undertaken at that time. On January 22, 1959, this same patient was again referred for bronchoscopy, this time by another chest physician. The second bronchoscopy showed the right upper lobe bronchus to contain broadened divisional spurs, the mucosa of which showed small punctate openings on telescopic examination, representing dilated bronchial gland ducts. At this time, probably because of a discharge of grayish pus, the areas of pitting within the left upper lobe wall were not seen, since no mention of these areas was made in the bronchoscopic operative note. Bronchography a few days later clearly demonstrated several extraluminal dye-filled shallow pockets within the floor of the left upper lobe bronchus, as illustrated in figure 4. Numerous other areas of dilated bronchial glands were seen in other branches, on the right and in the periphery, in the wall of the bronchi of the third and fourth generations.

Case 5. S. M., a 58-year-old male, was diagnosed as having severe obstructive emphysema, chronic suppurative bronchitis, and cor pulmonale. He was bronchoscoped on March 23, 1959. Telescopic visualization of the right upper lobe orifice showed close to its margin several mucosal holes measuring 1 to 1.5 mm. in diameter. These openings were interpreted as dilated bronchial glands. Telescopic inspection of the left upper lobe orifice and adjacent mucosa of the left main stem bronchus showed a single hole in the inferior wall of the left upper lobe bronchus, as well as multiple small openings in the inferior (posterior) wall of the intermediary left lower lobe bronchus. They also were interpreted as dilated bronchial gland openings. Bronchography a few days later not only demonstrated these telescopically seen openings, but numerous other extraluminal, dye-filled structures further in the periphery, giving the bronchial wall a frayed or serrated outline as shown in figures 5 and 6.

Thus, the aforementioned cases represent just a few examples of the similarity of the telescopically seen mucosal detail with the bronchographic pattern obtained by contrast filling of the dilated glands. In the accompanying illustrations some typical bronchographic findings are photographically reproduced. However, endoscopic photography of the telescopically seen openings was not available. Therefore an attempt has been made to sketch the endoscopic (telescopic) appearance of the frequently seen openings for photographic reproduction (figures 7 and 8).

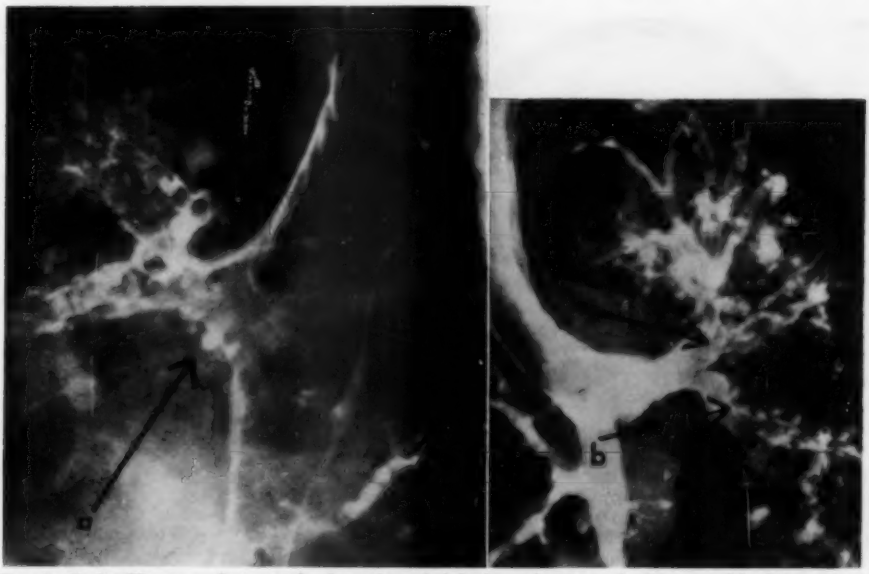

FIG. 5. (Left) Close-up photograph of portion of right bronchogram of a 58-year-old white male. (*a*) Arrow points to largest of several dye-filled dilated glands in floor of right upper lobe bronchus. (*b*) Arrow points to multiple areas of extraluminal dye-filling in a subdivision of the posterior branch, causing serrated outline. Other branches show the same peripheral pattern.

FIG. 6. (Right) Same patient as in figure 5. Portion of the left half of bronchogram shown. (*a*) Arrow points to dye-filled sac in floor of anterior division of left upper lobe bronchus. (*b*) Arrow points to multiple areas of bronchial gland dilatation located in superior wall of lingular division.

Those in the profession who use the right angle or forward-oblique telescope in their endoscopic work and all house staff members to whom the mucosal openings encountered during bronchoscopy were repeatedly shown will immediately recognize the veracity of a more concentrated accumulation of such mucosal gland openings. The illustrations in figures 7 and 8 may be considered as composites of what is quoted from the bronchoscopic operative reports of *Cases 2*, *3*, and *5* (figures 2, 3, 5, 6).

DISCUSSION

In the series of 250 cases bronchoscoped at the Church Home and Hospital only those patients underwent bronchographic study in whom more information was sought than could be obtained by bronchoscopy. Thus, the 33 bronchoscopies in which findings were proved by subsequent bronchography represent only about 40 per cent verification of endoscopic findings. There were only 5 cases in which bronchography failed to show the openings and sacs of the dilated bronchial glands seen previously and noted endoscopically. These bronchograms were poor in quality, either because of the use of an abundance of dye, poor anesthesia response, or asthmatic bronchial spasm, which interfered with distribution, flow, and progress of the dye. Also, motion on the part of the patient may have played a part. The remaining endoscopically visualized gland openings in 34 separate bronchoscopies were not followed by bronchography. It is believed that a 90 per cent verification of the endoscopic observations could be rendered by subsequent skillful bronchography.

A total of 77 demonstrations of dilated bronchial glands was rendered in the 250 bronchoscopy cases, or 31 per cent, if the 5 cases of bronchographic visualization without endoscopic visualization of the openings are included.

In table 3 may be noted the predominant disease present in the 77 cases which permitted visualization or demonstration of the dilated bronchial glands, sinus tracts, or pseudo-diverticula. The table is self-explanatory since the addition of all cases of bronchitis (9 or 12 per cent), asthma-allergic bronchitis (4 or 5 per

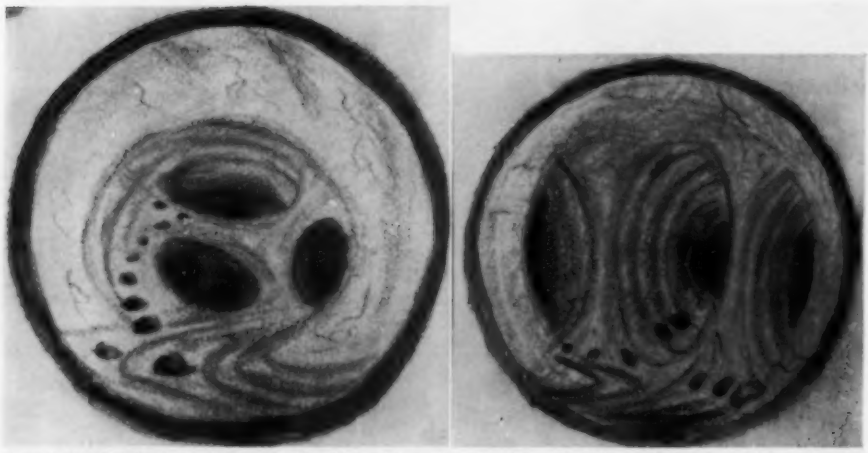

FIG. 7. (Left) Photographic reproduction of drawing showing the telescopic view of the right upper lobe bronchus. Of the three openings, the apical division leads off to the right, the anterior division upward, and the posterior division downward and to the left. Multiple areas of pitting are shown the largest beginning in the membranous portion of the main bronchus, the other openings extending in the floor of the upper lobe bronchus as far as into the divisional spur between the anterior and posterior divisions. The drawing could be considered a composite of what was seen endoscopically in *Cases 3* and *5*, corresponding to figures 3 and 5.

FIG. 8. (Right) Photographic reproduction of a drawing, showing the right angle telescopic view of the left upper lobe bronchus. The openings seen from left to right are: apico-posterior division, anterior division, and lingular division. A number of small mucosal openings are shown near the membranous folds in the initial portion of the floor (posterior wall) of the left upper lobe bronchus. Two openings of dilated bronchial glands are shown in the floor of the anterior division. The drawing can be considered a composite of the endoscopic visualization in *Cases 2*, *4*, and *5*, corresponding to the bronchograms shown in figures 2, 4, and 6.

cent), obstructive emphysema with bronchitis (21 or 27 per cent) and bronchiectasis, chronic lung abscess (15 or 20 per cent) amounts to 49 of the 77 demonstrations (64 per cent), approximately two-thirds of the total. Since cancer of the bronchus is frequently preceded by years of bronchitis and many patients with long-standing pulmonary tuberculosis have associated chronic bronchitis, the table in reality only confirms that the finding of dilated bronchial glands, whether by endoscopic visualization of the openings or by contrast filling during bronchography, is an important morphologic finding of a definite pathognomonic state, namely, chronic deforming bronchitis, a term used frequently in the literature elsewhere (5, 10).

Rayl and Smith (14) and most recently Freimanis and Molnar (15) have given a most concise description and explanation of the bronchographic demonstration of dye-filled extraluminal sacs in addition to the first cases reported in the United States literature (16). Their descriptions were based mainly on the previously enumerated extensive foreign literature in which Stutz (5, 10) stated that, with the exception of true bronchial diverticula, the bronchographic sacs and extraluminal areas of filling represented dilated hypertrophied and inflamed bronchial glands. Duprez and Mampuys (3) examined these glands histologically and found the apertures more frequently in the larger bronchi than in the trachea and more numerous in the neighborhood of bronchial bifurcations. Histologically they found these apertures to be the openings of long ducts which often branch off in the depth and frequently extend beyond the level of the cartilage ring. Freimanis and Molnar (15) reported that these gland ducts run through the muscular layer, that they turn sideways, and run almost parallel to the mucosal surface. They believe that this topographic arrangement encourages obstruction by thick mucous plugs which, in turn, lead to the

dilatation frequently seen in bronchographic demonstrations.

Reid (17) presented a detailed outline of the pathology of chronic bronchitis seen in bronchographic demonstrations. Bronchial gland dilatation was only one of many features. The possibility of dealing with an "anlage" is to be considered. Hayek (12), in his textbook *The Human Lung*, presented illustrations of diverticula originating in the wall of the mucous glands of the smaller bronchi. He demonstrated accumulations of lymphoid tissue around these diverticula and suggested the concept of a "pulmonary tonsil." The question under consideration here is the source of his anatomic material. Unless the anlage can be shown to exist in the newborn, there is a temptation to believe that diverticula-like enlargements of bronchial glands represent the inevitable response of the bronchial mucosa to the inhalation of modern-day smoke, fumes, dust, chemicals, and contents of the atmosphere to which all adults are exposed. Thus, if Hayek's description is derived from post-mortem examination of adult lungs, the findings would be classified as pathologic anatomic rather than normal anatomic findings. Since it is apparently more correct to speak of dilated bronchial glands, one can find ample argument for the use of the words diverticula (4, 9, 14) or diverticula of the bronchial glands, although the true bronchial diverticulum arises at the location of an embryonically possible origin of a bronchial division.

It is astounding that, despite many bronchographic demonstrations of dye-filled extraluminal sacs, bronchoscopies in such cases fail to show more than inflammatory swelling of the mucosa, coating with mucopurulent secretions, and areas of reddening (13, 16). Stutz and Vieten (10) stated emphatically that, in their numerous cases of bronchographic demonstration of dilated, inflamed bronchial glands, bronchoscopy could not corroborate this finding in a single instance, and they believed that the dilatations could never be demonstrated bronchoscopically. Obviously, the well-supported endoscopic visualization as first recorded by Duprez and Mampuys (3) is ignored. The latter writers were of the opinion that the openings of normal bronchial glands are hidden from endoscopic view in the normal or in an edematous mucosa. Also, a layer of mucus may cover and conceal them. On the other hand Duprez and Mampuys (3) pointed out that some of the histologic evidence indicated that inflammatory changes persist around dilated ducts and glands. They further believed it possible that these changes could play a role in reinfection of the lung in cases of long-standing bronchitis and in the maintenance of such infection which otherwise would have subsided. Endoscopic observation emphasized the fact that the vast majority of the dilated gland openings presented themselves as sharply punched-out holes without any inflammatory reaction surrounding these areas. Only occasionally could a drop of glassy secretion be seen hanging inertly in the opening. Rarely was there reddening of the floor of the confluent, larger openings or of the adjacent mucosa unless there was diffuse irritation of the entire region of one or both bronchi.

The present writer agrees with Duprez and Mampuys (3) as to the predilection of the endoscopically seen dilated gland openings, namely, at the bifurcation of the upper lobe bronchi, the adjacent ridges of the membranous posterior wall of the main bronchi, as well as at the junction of the membranous and cartilaginous portions of the bronchi. Most frequently the openings were seen in the floor (topographically, the posterior portion) of the upper lobe bronchi. This may be caused by better telescopic accessibility of these regions, since it is infinitely more difficult to visualize the roof or anterior portion of the upper lobe orifices and bronchi. Yet, the respective bronchograms often show dilated bronchial glands in the superior or anterior walls (*see* figure 6, arrow *b*). Finally, it must be realized that the right-angle telescope is used primarily to visualize the divisions of the upper lobe bronchi, a view "around the corner," so that the frequency of discovery of pitted areas, holes, and bigger gland openings may actually depend on the magnification afforded in exploring these regions. It may even be postulated that a fair number of such openings might be discovered elsewhere in the tracheobronchial junction and the walls of both main stem bronchi if a search were systematically made of the entire circumference of the respective bronchi. This, at best, would be time-consuming and even tedious, and would prolong unnecessarily the bronchoscopic procedure, possibly to the disadvantage of the patient. Thought should be given to such a systematic search in selected bronchoscopies in which the

patient's condition allows for some prolongation of instrumentation.

Summary

Endoscopic visualization of the openings of dilated bronchial mucous glands is a fairly frequent and heretofore neglected morphologic finding. Since these dilated glands recently have been demonstrated roentgenographically by bronchography in increasing numbers, a review was made of two series of bronchoscopies, one comprising 896 bronchoscopies on 654 patients at a tuberculosis sanatorium, where positive endoscopic findings were observed 94 times in 72 patients, an 11 per cent incidence. The other series covered 250 bronchoscopies in a general hospital, with demonstration of the openings of dilated bronchial glands in 72 instances (29 per cent). There were 33 cases in which subsequent bronchography confirmed the presence of dilated bronchial glands at the site indicated in the preceding bronchoscopy. The literature concerning the occurrence, pathogenicity, and significance of this morphologic finding is reviewed. The necessity for using the telescopic magnification for visualization of this mucosal detail is emphasized. Figures are given with respect to the bronchopulmonary diagnosis in the general hospital series. These indicate that the frequency of discovery of the previously neglected morphologic sign is directly proportional to the existence of some form of chronic bronchitis. It is believed that the endoscopic visualization should be confirmed and noted as frequently by the endoscopist as the bronchographic demonstration of the dilated glands is reported in roentgenograms. Five cases are cited with bronchographic reproductions. Two endoscopic views are presented in drawings and are believed to be the first shown in the literature.

Sumario

Dilatación de las Glándulas Mucosas Bronquiales en la Bronquitis Crónica, Hallazgo Morfológico Descuidado. Correlación del Aspecto Broncoscópico y Broncográfico

La visualización endoscópica de las aberturas de las glándulas mucógenas bronquiales dilatadas constituye un hallazgo morfológico bastante frecuente y menospreciado hasta ahora. Visto que estas glándulas dilatadas han sido observadas radiográficamente en números cada vez mayores en la broncografía, se verificó un repaso de dos series de broncoscopias, comprendiendo una 896 broncoscopias en 654 enfermos de un sanatorio para tuberculosos, en la que se notaron hallazgos endoscópicos positivos 94 veces en 72 enfermos, o sea una incidencia de 11 por ciento. La otra serie comprendió 250 broncoscopias en un hospital general, observándose las aberturas de glándulas bronquiales dilatadas en 72 casos (29 por ciento). Hubo 33 casos en los que la broncografía subsecuente confirmó la existencia de glándulas bronquiales dilatadas en el sitio indicado por la broncoscopia anterior. Se repasa la literatura relativa a la ocurrencia, patogenicidad e importancia de este hallazgo morfológico, recalcándose que es necesario usar el aumento telescópico para la visualización de este detalle mucoso. Se ofrecen cifras con respecto al diagnóstico broncopulmonar en la serie del hospital general, indicando las mismas que la frecuencia con que se descubre el signo morfológico antes descuidado guarda razón directa con la existencia de alguna forma de bronquitis crónica. Opínase que la visualización endoscópica debe ser confirmada y notada por el endoscopista con la misma frecuencia con que se observa en las radiografías el hallazgo broncográfico de la dilatación de las glándulas. Se citan 5 casos con reproducciones broncográficas. En los diagramas se presentan dos vistas endoscópicas que parecen ser las primeras mostradas en la literatura.

Resume

Glandes bronchiques à mucus dilatées dans la bronchique chronique, une donnée morphologique négligée. Corrélation entre l'apparence bronchoscopique et bronchographique

La visibilité endoscopique des ouvertures des glandes bronchiques muqueuses dilatées est trouvée assez fréquemment, et, par conséquent est une donnée morphologique négligée. Depuis qu'on a récemment montré, de plus en plus, ces glandes dilatées, radiologiquement par bronchographie, on a passé en revue deux séries de bronchoscopies, une comprenant 896 bronchoscopies de 654 malades, dans un sanatorium pour tuberculose où l'on observa des présences endoscopiquee positives 94 fois chez 72 malades, une fréquencs de 11%. Les autres séries couvrirent 250 bronchoscopies dans un hôpital général, avec la mise en évidence des ouvertures des glandes bronchiques dilatées dans 72 cas (29%). Dans 33 cas, une bronchographie ultérieure confirma la présence de glandes bronchiques dilatées à l'endroit indiqué par la précédente bronchoscopie. La littérature concernant la fréquence, la pathogènie et la signification de ces données morphologiques est

passée en revue. Les auteurs insistent sur la nécessité d'employer l'agrandissement telescopique pour rendre visible les détails de la muqueuse. Ils donnent des chiffres se rapportant au diagnostic bronchopulmonaire dans les séries de l'hôpital général. Ceux-ci indiquent que la fréquence de la découverte de ce signe morphologique auparavant négligé, est directement proportionnelle à l'existence d'une forme quelconque de bronchite chronique. On croit que la visibilité endoscopique est à confirmer et à noter par l'endoscopiste, aussi fréquemment que par la démonstration bronchographique des glandes dilatées relatées par les radiographies. Les auteurs citent cinq cas avec des reproductions bronchographiques. Ils présentent deux vues endoscopiques dessinées qui sont, croit-on, les premières montrées dans la littérature.

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THE DIAGNOSIS OF ACUTE PULMONARY EMBOLISM BY ARTERIOGRAPHY[1, 2]

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Introduction

The Trendelenburg operation for removal of a pulmonary embolus has been almost completely abandoned due to the high mortality rate associated with this procedure and the difficulty in differentiating thrombo-embolism from other acute circulatory accidents. A reliable diagnostic procedure would permit selection of suitable patients for embolectomy without fear of error This study was undertaken to evaluate pulmonary arteriography as a diagnostic procedure in experimental pulmonary embolism.

In 1908, Trendelenburg (1) described a method of pulmonary embolectomy and reported three clinical cases; all of the patients died during the procedure or a few hours after the embolectomy. Despite these failures, Trendelenburg demonstrated that, in selected cases of pulmonary embolism, a sufficient time interval exists for embolectomy. Kirschner (2), in 1924, reported the first successful pulmonary embolectomy, and the procedure was attempted many times in the Scandinavian and European countries during the next twenty years (3). Only twelve successful cases were reported during this period. The only successful pulmonary embolectomy in this country was reported by Steenburg in 1958 (4).

Experimental Method

A series of twenty adult mongrel dogs were anesthetized with intravenous sodium pentobarbital; continuous arterial and venous pressures and electrocardiograms were recorded. Employing a portable X-ray unit, a single-exposure control pulmonary arteriogram was obtained by injection of 20 ml. of 50 per cent diatrizoate (Hypaque®) into the external jugular vein (figure 1). A large thrombus was formed by injecting topical thrombin into an occluded segment of the inferior vena cava. The thrombus was released, and lodgement in the pulmonary artery was judged by a transient fall in blood pressure and, occasionally, a rapid increase in respiratory rate. A second arteriogram was then obtained and the presence and location of the embolus was clearly demonstrated (figures 2, 3, 4). A pulmonary embolectomy was performed as rapidly as possible, utilizing an arteriotomy in the main pulmonary artery in central emboli and an arteriotomy at the interlobar fissure in peripheral emboli (figure 5).

Results

The arteriogram demonstrated an embolus in 17 of the 20 animals. In one instance a large embolus which filled the entire pulmonary artery and occluded all flow was not demonstrated. The other two failures were due to faulty timing of the arteriogram. In the remaining 17 animals, the embolus was clearly demonstrated. Small emboli occluding lobar arteries, large emboli occluding the entire pulmonary artery, and multiple emboli were all demonstrated by this method. Blood pressure and electrocardiographic changes were not specific in establishing the diagnosis. A depressed S-T segment and inverted T wave in lead I were observed in only 3 instances. Persistent hypotension occurred only when both pulmonary arteries were occluded. A pulmonary embolectomy was performed in each instance, utilizing the main pulmonary artery or an interlobar artery as indicated. Twelve of the animals (60 per cent) survived embolectomy; the failures were due to cardiac arrest, blood loss, and incomplete embolectomy.

Discussion

It has been shown in clinical studies that in fatal pulmonary embolism one half of the patients lived fifteen minutes or longer after the initial symptoms (5). A sufficient time interval exists, therefore, to permit pulmonary embolectomy in selected situations. Physical findings of sudden dyspnea, tachycardia, hypotension, and distended neck veins are suggestive of pulmonary embolism but are not absolutely diagnostic. Electrocardiographic changes are helpful but are diagnostic only when serial tracings can be obtained. As a result, there is usually not sufficient

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[2] Presented before the Medical Session, as part of *Section 7A*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 18, 1960.

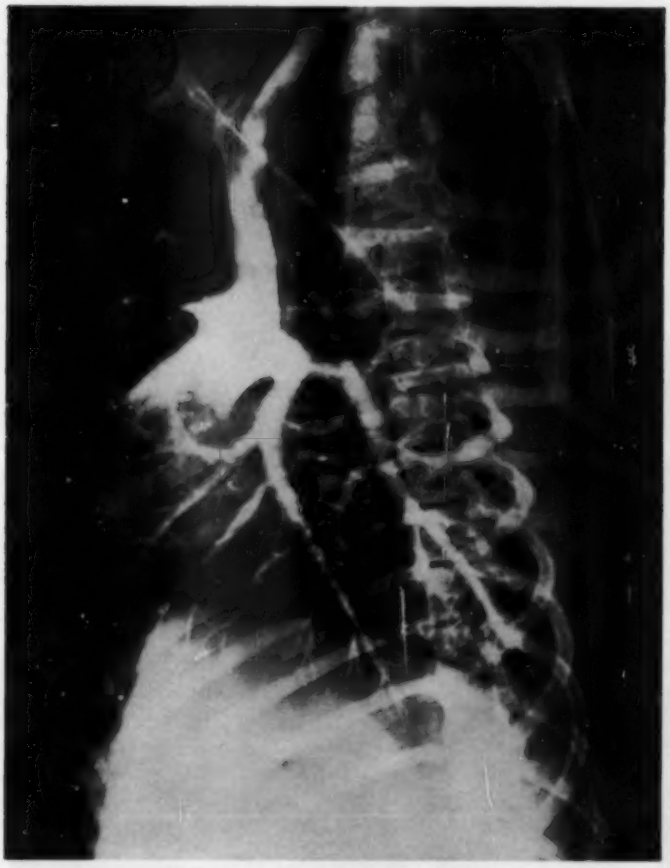

FIG. 1. Control arteriogram made in the right posterior oblique position. The superior vena cava, right ventricle, and right and left pulmonary arteries are visualized.

evidence to warrant the rather formidable operative procedure of pulmonary embolectomy.

Angiocardiograms performed by the intravenous injections of large amounts of concentrated contrast medium, using multiple roentgenographic exposures, have been found to be both safe and practical. Frequently, an incidental pulmonary arteriogram is visualized in the early exposures and is seen on two or more films, depending on the timing. Usually the pulmonary artery is best visualized 2.5 to 3.5 seconds after the onset of the injection (figure 6). This rather complicated X-ray technique with its precise timing is easily modified to a single-exposure method using a portable X-ray unit at the bedside similar to the technique adopted for experimental animals. Fifty cubic centimeters of 90 per cent diatrizoate is injected intravenously through a large-bore needle as rapidly as possible. A single exposure made one second after the completion of the injection should give visualization of the pulmonary artery (6).

Utilizing this method, a pulmonary embolus might be demonstrated by arteriography in a relatively short period of time, using readily available equipment. Application of this diagnostic procedure to selected clinical situations would arm the surgeon with knowledge of the presence and exact location of the embolus. Errors in diagnosis could be avoided, and em-

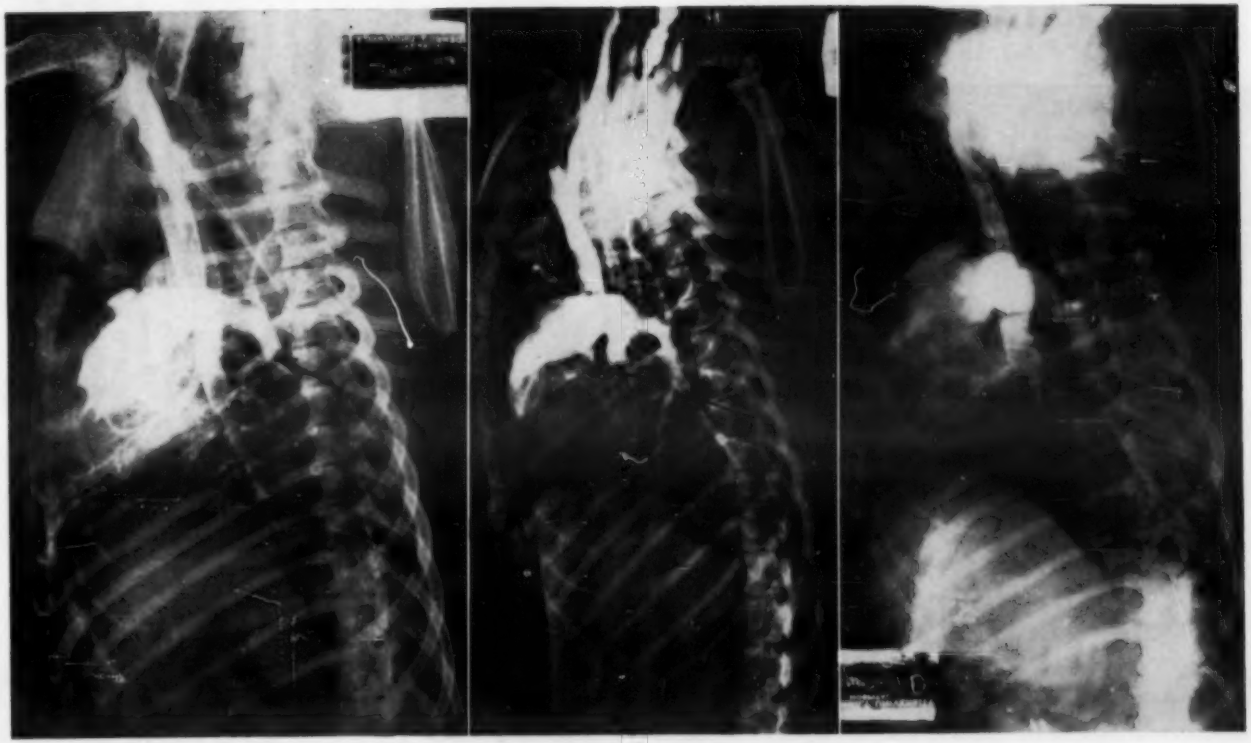

FIG. 2 (Left). The left pulmonary artery is completely occluded by an embolus.
FIG. 3 (Center). The right pulmonary artery is completely occluded. A second smaller embolus is seen in the distal portion of the left pulmonary artery
FIG. 4 (Right). The right pulmonary artery is completely occluded. A filling defect is seen in the distal portion of the left pulmonary artery.

FIG. 5. Emboli removed from the animal represented in figure 4. The large embolus was removed from the right pulmonary artery and the small embolus produced the filling defect seen in the left pulmonary artery.

bolectomy would be directed toward a specific site rather than an exploration of the entire pulmonary artery.

These factors should permit revival of the Trendelenburg operation in selected patients, and proper application should improve the mortality rate of this highly lethal condition.

SUMMARY

A pulmonary embolus was produced in each of 20 dogs by injection of topical thrombin into the vena cava. A single-exposure, portable X-ray arteriogram was successful in demonstrating the location of the embolus in 17 of the animals. Pulmonary embolectomy was successful in 60 per cent of these animals. Clinical application of pulmonary arteriography is proposed in selected patients with pulmonary embolism.

SUMARIO

El Diagnóstico de la Embolia Pulmonría Aguda por la Arteriografía

En cada uno de 20 perros se produjo un émbolo pulmonar con la inyección de trombina tópica en la vena cava. Un arteriograma roentgenográfico tomado con una sola exposición en un aparato portátil bastó para revelar la localización del émbolo en 17 de los animales. La embolectomía pulmonar surtió efecto en 60 por ciento de estos animales. Se propone la aplicación clínica de la arteriografía pulmonar en enfermos seleccionados que padezcan de embolia pulmonar.

RESUME

Le diagnostic par artériographie dans l'embolie pulmonaire aiguë

On produit une embolie pulmonaire chez chacun des 20 chiens en injectant de la thrombine topique dans la veine cave. Un artériogramme, fait avec un appareil portatif à exposition unique, put montrer avec succés l'endroit de l'embolie chez 17 des animaux. Une embolectomie pulmonaire fut pratiquée avec succés chez 60% de ces animaux. Les auteurs proposent l'application clinique de l'artériographie pulmonaire chez des malades sélectionnés ayant une embolie pulmonaire.

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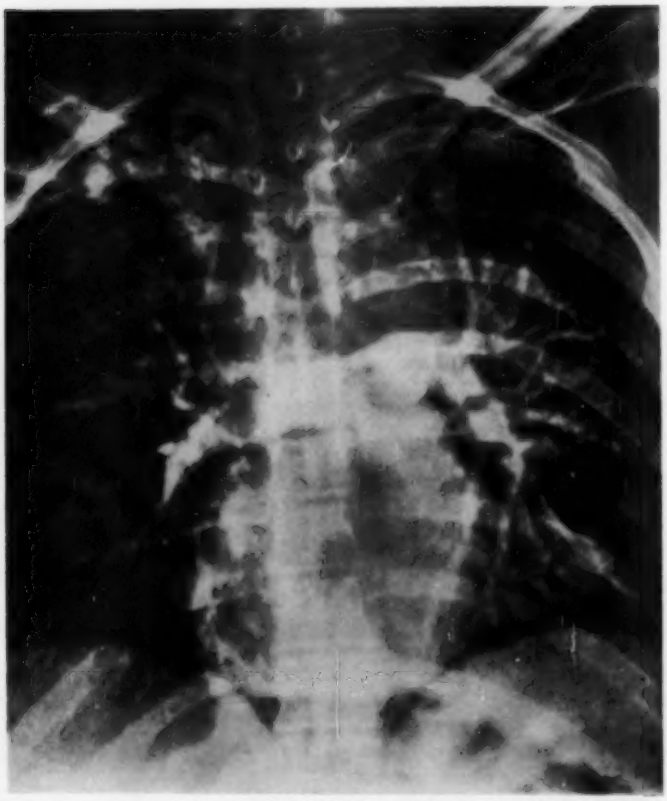

FIG. 6. A normal pulmonary arteriogram obtained 2.5 seconds after intravenous injection of 50 ml. of 90 per cent diatrizoate. This patient had a large pericardial cyst.

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BRONCHIAL ARTERIAL COLLATERAL CIRCULATION[1, 2, 3]

Effect of Experimental Ligation of the Pulmonary Artery and Subsequent Reanastomosis

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INTRODUCTION

Liebow and associates (1) have summarized our knowledge of the anatomy and physiology of the dual circulation of the lung. In the normal lung, the bronchial arteries, branches of the systemic circulation, are small and serve as nutrient vessels to the bronchi out to the level of the respiratory bronchioles. The large pulmonary arteries transport venous blood to the pulmonary capillary bed for respiratory gas exchange. This clear division in anatomic and functional relationships of the two systems is radically altered in certain pathologic states.

An expansion of the bronchial arterial collateral circulation and the development of precapillary communications with the pulmonary artery bed are observed under two general conditions: when pulmonary disease states lead to lung destruction and healing by organization; and when the pulmonary artery pressure is reduced, as in severe pulmonary stenosis. A simulation of the vascular changes observed in the latter can be experimentally induced by ligation of the pulmonary artery in the dog and other laboratory animals.

As summarized by Liebow and associates (1), experimental studies employing dogs which have had the pulmonary artery to one lung ligated support the following opinions. The large collateral circulation induced by ligation of the pulmonary artery is greater when the artery is interrupted in the newborn than in the adult animal. In the absence of infection, precapillary communications between the bronchial and pulmonary circulations in the order of 50 μ appear. (Much larger bronchial-pulmonary arterial communications are observed in chronic suppurative pulmonary disease. For example, communications exceeding 1 mm. in size are often seen in advanced bronchiectasis.) In the experimental dog, the minimal collateral arterial flow has been observed to be in excess of 100 ml. per minute within two weeks after ligation of the pulmonary artery and approaches 800 ml. per M^2 per minute by eighteen months. The pressure in the pulmonary artery distal to the ligature remains lower than the pressure in the pulmonary artery trunk. When the pulmonary veins draining a lung with expanded bronchial collaterals are acutely obstructed, the pressure in the pulmonary artery distal to the ligature rises to approach the mean systemic arterial pressure. Bronchial arteriography reveals rapid filling of the pulmonary artery distal to the ligature followed by visualization of the pulmonary veins. This bronchopulmonary shunt has also been demonstrated by indicator dye dilution curves.

The purpose of the present report is to present data which shed further light on two of the many unanswered questions relating to the genesis and functional significance of the collateral circulation of the lung. These are: the anatomic fate of experimentally expanded bronchial arterial collaterals following reanastomosis of the ligated pulmonary artery; and the effect of restoration of pulmonary arterial continuity upon the distribution of bronchial arterial blood flow.

METHODS

Adult mongrel dogs were the subjects of the present experiments. Surgical and angiographic procedures were performed under sodium pentobarbital anesthesia (30 mg. per kg.), employing diatrizoate (Hypaque®) as contrast material.

Operative technique: Simple ligation of the pulmonary artery to one lung is relatively easy to perform and is well tolerated by healthy dogs. On the other hand, reanastomosis of a pulmonary artery several months following ligation poses enormous technical difficulties. These difficulties are caused by the vascularity of the field which presents a plexus of fragile bronchial collaterals and because the pulmonary artery distal to the

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[2] This study was supported by Project H-1927, National Institutes of Health, U. S. Public Health Service.

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ligature becomes atrophied in length, caliber, and wall thickness. After many unsuccessful attempts to reanastomose the chronically ligated pulmonary artery in dogs, the importance of certain technical considerations became apparent. The operative procedure which evolved reduced the operative mortality from almost 100 to 25 per cent.

The left pulmonary artery was ligated transpericardially at its origin, employing a median sternotomy incision for entry into the chest. The left pulmonary artery was chosen because it is anatomically more accessible than the right, which is partially obscured by the ascending aorta. Ligation of the artery flush with its origin preserves the maximal length of the vessel distal to the ligature, a factor of critical importance when reanastomosis at a later date is contemplated. A median sternotomy approach reduced to a minimum the development of pleural adhesions which only add to the difficulties of the secondary procedure.

Reanastomosis of the ligated pulmonary artery was accomplished through a left thoracotomy, employing the fourth intercostal space for entry into the chest. Significant damage to the bronchial collaterals can be avoided by restricting the dissection of the ligated pulmonary artery to its intrapericardial portion. With the aid of suitable vascular clamps, the ligated origin of the left pulmonary artery was excised and reanastomosed to an opening of corresponding caliber in the pulmonary artery trunk. Careful vascular technique employing fine arterial sutures is essential.

Anatomic studies: Gross anatomic changes were recorded by the preparation of bronchovascular corrosion casts after a method adapted from Liebow and co-workers (2). To Batson's injection mass, as described by Wolfe (3), lead carbonate and vinylite were added in the following proportions:

Justi #17 corrosion base, 2,000 ml.
Lead carbonate powder, 500 gm.
Vinylite powder, 100 gm.

Angiography: Three types of angiographic studies were employed: venous angiocardiography, bronchial arteriography by the method of Nordenström (4), and bronchial arteriography performed by direct cannulation of a branch at open thoracotomy. The method of Nordenström, which employs cannulation of the right sixth intercostal artery with the chest intact, proved successful in the present study only twice in 11 attempts. Direct cannulation of a bronchial arterial branch for angiography, with the chest open and the dog connected to a respirator, was technically satisfactory, and gave results similar to those obtained in the two successful bronchial arteriograms performed by the method of Nordenström. Bronchial arteriograms by the open cannulation technique were made in 8 dogs immediately following reanastomosis of the pulmonary artery and with temporary occlusion of the vessel by a clamp. The duration of pulmonary arterial obstruction prior to reanastomosis varied from four months to two years prior to these studies.

Observations

Bronchial Circulation following Pulmonary Artery Ligation

A series of 12 dogs subjected to ligation of the left main pulmonary artery served as controls. These animals were sacrificed at intervals ranging from two months to two years following ligation of the pulmonary artery. Bronchovascular corrosion casts of the lungs were prepared for study by injection *in situ*. Anatomic confirmation of Liebow's description of the exuberant expansion of the bronchial arterial collaterals, which occurs after pulmonary artery ligation, was uniformly obtained. Approximately 80 per cent of the expansion occurred within three months following ligation of the pulmonary artery. Retrograde filling of the branches of the pulmonary artery distal to the ligature occurred only once in this series, but this is probably a function of the relatively greater viscosity of the mass employed by the present writers for the preparation of bronchovascular casts in comparison with the injection mass employed by Liebow.

Bronchial Circulation following Reanastomosis of a Ligated Pulmonary Artery

Twenty-two dogs survived technically satisfactory reanastomosis of the ligated left pulmonary artery. Of these, 17 animals developed thrombosis at the site of the anastomosis, probably within hours following surgery, as demonstrated by early postoperative venous angiocardiography. These 17 animals were sacrificed at intervals up to eighteen months, and no attempt at recanalization of the thrombosed site of anastomosis was observed. The thrombus organized and was replaced by an occlusive scar. The artery distal to the stricture remained patent and the vascular changes were the same as in the control animals.

The 5 dogs in which the pulmonary artery anastomosis remained patent are of special interest. Four of these had strictural narrowing at the anastomotic site, and exhibited persistence of expanded bronchial collaterals. The duration of ligation of the pulmonary artery prior to reanastomosis in these four dogs was four and one half to eighteen months. A corresponding interval for

each animal followed prior to sacrifice and preparation of bronchovascular casts. The lumen of the stricture at the site of reanastomosis measured one-fourth to one-eighth that of the opposite pulmonary artery (figure 1*A*). Despite the persistence of bronchial arterial collaterals and the pulmonary artery stricture on the left, angiocardiography (figure 1*B*) revealed filling of the pulmonary arterial systems of both lungs.

The fifth dog of this group underwent reanastomosis of the left pulmonary artery two months after ligation. At operation a greatly expanded plexus of bronchial arterial collaterals was observed. In contrast to the observation in dogs with pulmonary artery ligation of longer than three months duration, it was noted that the left pulmonary artery distal to the ligature had undergone very little atrophy. At sacrifice seventeen months later the bronchial circulation, the caliber of the pulmonary vascular elements, and the gross size of the two lungs were normal. Regression of the exuberant bronchial arterial collaterals to normal in this animal appeared to be related to the relatively short duration of ligation of the pulmonary artery and the absence of stricture at the anastomotic site.

To test this hypothesis two dogs with ligated left pulmonary arteries of 4 and 5 months' duration, respectively, were subjected to reanastomosis of the ligated pulmonary artery. In addition, however, the contralateral pulmonary artery was tied down with umbilical tape to approximate the caliber of the treated pulmonary artery which had attenuated to half the caliber of its fellow. Large bronchial arterial collaterals were present (figure 2*A*, *B*). Postoperative angiocardiograms revealed filling of both pulmonary arterial systems (figure 2*C*). At sacrifice eleven months later, the bronchial arterial collaterals had regressed to normal in one, and the other exhi-

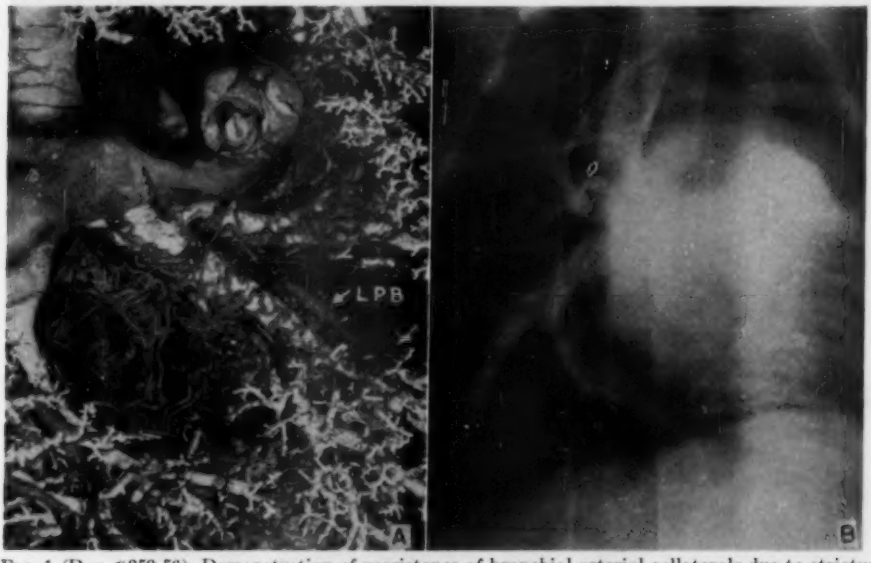

FIG. 1 (Dog #350-56). Demonstration of persistence of bronchial arterial collaterals due to stricture at site of left pulmonary artery reanastomosis. The duration of pulmonary artery ligation was five months. The studies shown were made five months following reanastomosis.

A. Bronchovascular cast: Anterior view of tracheal bifurcation showing immense plexus of bronchial arterial collaterals coursing over the left hilum. LPB: left pulmonary artery branch. The left main pulmonary artery and stricture which measured one-fourth the diameter of the right pulmonary artery are hidden from view. Cast shows retrograde filling through bronchial-pulmonary artery communications which cannot be discerned in this black-and-white photograph. The pulmonary veins are not injected.

B. Angiocardiogram taken prior to sacrifice reveals simultaneous visualization of the pulmonary arteries of both lungs. Note also that the left lung and pulmonary artery system are much smaller than those on the right.

FIG. 2 (Dog #220-57). Pulmonary artery continuity restored after five months of ligation. Caliber of pulmonary arteries equalized by constriction of opposite pulmonary artery with umbilical tape. Animal sacrificed eleven months later and regression of expanded bronchial arteries demonstrated.

A. Anterior view of bronchovascular cast showing normal relative size of the two lungs. The pulmonary veins are not injected.

B. Close-up of hilar area. Note equal size of left and right pulmonary arteries at the level of anastomosis and constriction, respectively. Contrast the bronchial artery trunk distributed to the left hilum with the plexus present in figure 1.

C. Angiocardiogram showing normal pulmonary arterial filling just prior to sacrifice.

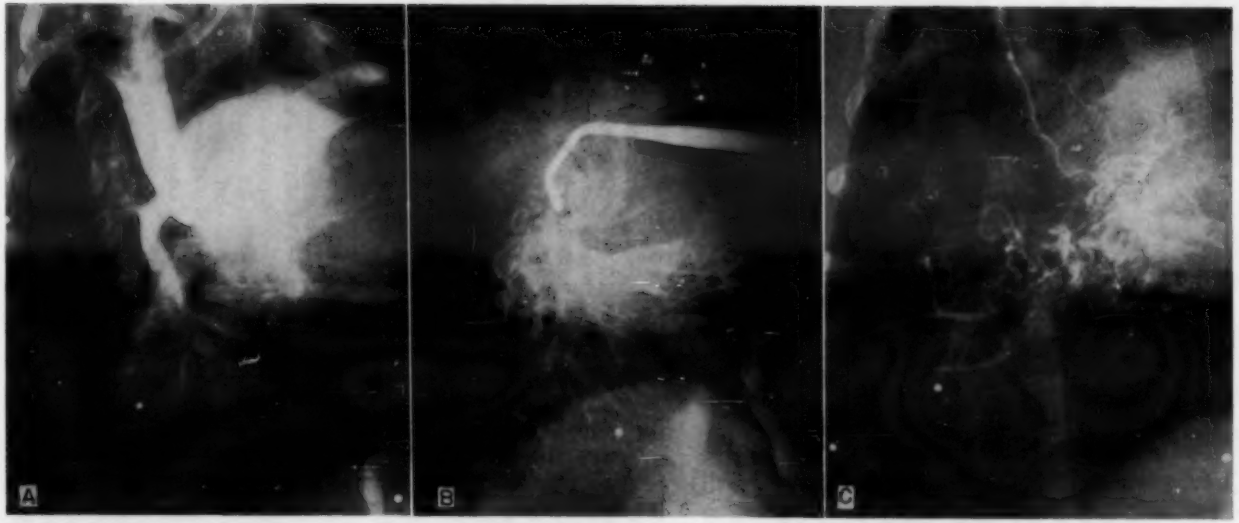

FIG. 3 (Dog #184-58). Studies immediately following reanastomosis of a left pulmonary artery which had been ligated for seven months.

A. Venous angiocardiogram showing simultaneous filling of the pulmonary arteries of both lungs.

B. Bronchial arteriogram with the reanastomosed left pulmonary artery occluded. Exposure taken two seconds following initiation of injection of 50 per cent diatrizoate into a cannulated bronchial artery branch. Note retrograde filling of the pulmonary artery branches to the point of occlusion.

C. Bronchial arteriogram repeated with the left pulmonary artery unoccluded. The material in this circumstance drains via the veins.

bited a slightly enlarged bronchial arterial branch distributed solely to the left hilum. Moreover, at the time of sacrifice, both lungs and the pulmonary vessels had returned to normal relative size.

It would appear, therefore, that regression of expanded bronchial arterial collaterals is dependent, not only upon restoration of blood flow through the restored pulmonary artery, but that the blood flow must be restored at a critical level of pressure.

Bronchial arteriography: Bronchial arteriograms, either by the method of Nordenström or by direct cannulation of a branch of the collateral system by open thoracotomy in dogs with induced bronchial arterial collaterals, revealed almost immediate retrograde filling of the ligated pulmonary artery to the point of ligature. This was followed in a few seconds by visualization of the pulmonary veins. This observation confirms those of Vidone and associates (5) and Nordenström (4). The pattern of this circulation was the same whether the bronchial arteriogram was performed according to the method of Nordenström or by direct cannulation of a bronchial arterial branch by open thoracotomy with controlled ventilation.

In contrast, following restoration of continuity in the ligated pulmonary artery, contrast material injected into the bronchial arteries did not opacify the larger branches of the pulmonary arterial system but drained by the pulmonary veins. This correlates with the demonstration of filling of both pulmonary arterial systems following reanastomosis of the pulmonary artery when contrast material is introduced by systemic veins for angiocardiography (figure 3).

Summary

Following ligation of the pulmonary artery, the bronchial arterial collaterals on the side of the ligated vessel become greatly expanded. This expansion is accompanied by the development of small precapillary communications between the two systems in the periphery of the lung. Although these changes develop progressively and reach a peak in eighteen months to two years, most of the increase in collateral occurs within three months following the ligation of the pulmonary artery. These changes, previously described, were again confirmed by the control animals herein reported.

The present study was undertaken to determine the long-term fate of expanded bronchial arterial collaterals following surgical restoration of continuity in the ligated pulmonary vessel and to determine the immediate effect of reanastomosis of the pulmonary artery on the pattern of bronchial blood flow.

The observations in the present study demonstrate the following: Experimentally induced bronchial collaterals will revert to normal provided the lumen of the reunited pulmonary artery is approximately the same size as that of the opposite pulmonary artery; marked narrowing at the site of reanastomosis of the pulmonary artery will result in persistence of the bronchial arterial collaterals; the pressure transmitted across the anastomotic bridge of the bronchial pulmonary artery is insufficient to cause reversed filling of the pulmonary artery after continuity of the artery has been restored. This was shown by angiocardiography and bronchial arteriography.

Sumario

Circulación Broncoarterial Colateral. Efecto de la Ligadura Experimental de la Arteria Pulmonar y la Reanastomosis Subsiguiente

Consecutivamente a la ligadura de la arteria pulmonar, se expanden considerablemente las ramas colaterales de las arterias bronquiales del lado del vaso ligado. Esta expansión va acompañada del establecimiento de pequeñas comunicaciones precapilares entre los dos sistemas en la periferia del pulmón. Aunque estas alteraciones aparecen gradualmente y alcanzan su cúspide en término de dieciocho meses a dos años, la mayor parte del aumento de la circulación colateral tiene lugar en término de tres meses de la ligadura de la arteria pulmonar. Estas alteraciones, previamente descritas, fueron confirmadas de nuevo en animales testigos según se describe ahora.

El estudio actual fué emprendido para determinar el destino a largo plazo de las ramas colaterales expandidas de las arterias bronquiales después del restablecimiento quirúrgico de la continuidad del vaso pulmonar ligado y para determinar el efecto inmediato de la reanastomosis de la arteria pulmonar sobre el patrón de la circulación sanguínea en los bronquios.

Las observaciones realizadas en el estudio actual demuestran lo siguiente: las ramas bronquiales colaterales inducidas experimentalmente volverán a lo normal con tal que la luz de la arteria pulmonar reunida sea aproximadamente del mismo tamaño que la arteria pulmonar opuesta; una estenosis acentuada en el sitio de la reanastomosis de la arteria pulmonar dará por resultado la persistencia de las ramas colaterales de las arterias

bronquiales; la presión transmitida a través del puente anastomótico creado por la arteria pulmono-bronquial es insuficiente para lograr la repleción invertida de la arteria pulmonar después de restablecer la continuidad de la misma. Esto quedó demostrado con la angiocardiografía y la arteriografía bronquial.

Resume

Circulation artérielle bronchique collatérale. Effet de la ligature expérimentale de l'artère pulmonaire et sa réanastomose consécutive

Après la ligature de l'artère pulmonaire, les collatérales artérielles bronchiques devinrent plus importantes du coté du vaisseau ligaturé. Cette expansion s'accompagna du développement de petites communications précapillaries entre les deux systèmes, à la périphérie du poumon. Bien que ces changements se développèrent progressivement et atteignirent leur maximum de dix-huit mois à deux ans, la plus grande augmentation des collatérales se produisit dans les trois mois qui suivirent la ligature de l'artère pulmonaire. Ces changements, précédemment décrits, furent à nouveau confirmés par les animaux de contrôle ici rapportés.

L'étude présente fut entreprise pour déterminer le destin à long terme des collatérales artérielles bronchiques augmentées après la restauration chirurgicale de la continuité dans le vaisseau pulmonaire ligaturé, et pour déterminer l'effet immédiat de la réanastomose de l'artère pulmonaire sur le mode de flot du sang bronchique.

Dans l'étude présente, les observations démontrent les faits suivants: les collatérales artérielles bronchiques suscitées expérimentalement retourneront à la normale, pourvu que la lumière de l'artère pulmonaire reconstituée ait approximativement la même dimension que l'artère pulmonaire opposée; un rétrécissement notable au niveau de la réanastomose de l'artère pulmonaire résultera en la persistance des collatérales artérielles bronchiques; la pression transmise par le pont anastomotique de l'artère pulmonaire bronchique est insuffisante pour causer un reflux envahissant l'artère pulmonaire, après que la continuité de l'artère a été restaurée. Ceci a été mis en évidence par angiocardiographie et par artériographie bronchique.

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A STUDY OF VIRUSES IMPLICATED IN ACUTE RESPIRATORY DISEASE IN CHICAGO FROM OCTOBER, 1958, to JUNE, 1959[1,2]

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Introduction

In the past decade a large number of new viruses have been isolated from the respiratory tract of man. Several of these have been associated with respiratory illness in children (adenoviruses, para-influenza 1, 2, and 3, chimpanzee coryza agent) (1–4). The importance of these new viruses in relation to acute respiratory disease in adults is still unknown. Adenoviruses have been associated with an epidemic disease of military recruits, but this infection is not common in the civilian population (1). The present report is concerned with attempts to isolate viruses from persons presenting clinical illnesses varying from mild afebrile common colds to classical influenza. From October 1, 1958, to June 1, 1959, throat swabs and acute and convalescent serum specimens were collected from the following sources at the University of Chicago Clinics: students reporting to the Student Health Service; patients admitted to the hospital; patients seen in outpatient clinics; and staff members and their dependents. Two hundred and thirty-seven specimens were collected, 228 of these from adults, 9 from children less than twelve years old.

Materials and Methods

Isolation of virus: Broth in which throat swabs were immersed was inoculated into tissue cultures of monkey kidney and Hep-2 cells. About half the specimens were inoculated without prior freezing. The monkey kidney cultures[3] were maintained on Medium 199 with 0.5 per cent calf serum. The Hep-2 line was grown on Eagle medium with 20 per cent human serum and maintained in Eagle medium, 5 per cent chicken serum. All tubes were examined for cytopathogenic effect three times a week, and hemadsorption tests with 0.4 per cent washed guinea pig erythrocytes were carried out at five- to seven-day intervals with monkey kidney cultures (5). Negative cultures were carried through at least two passages before being discarded.

Selected throat swabs were also inoculated into the amniotic cavity of ten- to twelve-day-old chick embryos for isolation of influenza viruses (6). After three days' incubation at 33°C. the eggs were opened and the amniotic fluid was removed and tested for the presence of hemagglutinin with 0.5 per cent washed chicken erythrocytes. Negative fluids were passed again intra-amniotically while positive fluids were injected intra-allantoically. Fluids negative on second intra-amniotic passage were discarded. Allantoic fluid from positive eggs was used for identification of virus.

Identification of viruses isolated in tissue culture: Influenza A and B were identified by hemagglutinin inhibition tests with known ferret convalescent antisera for A2/Jap/305/57 virus and B/GL/54 virus (6). Para-influenza 1 (hemadsorption type 2) and para-influenza 2 (croup-associated virus) were identified by means of the hemadsorption inhibition test on monkey kidney tissue cultures, using antiserum obtained from Dr. Robert Chanock of the National Institutes of Health (7).[4] Chimpanzee coryza agent and herpes simplex were identified by using infected fluids from tissue cultures as unknown antigens in complement-fixation tests with known antisera. The antisera used to identify chimpanzee coryza agent were acute and convalescent serum specimens from one of the persons from whom this virus was isolated. The acute specimen had an antibody titer of less than $\frac{1}{8}$; the convalescent a titer of $\frac{1}{64}$. These titers were confirmed by complement-fixation tests in the laboratories of Dr. Robert Chanock. The herpes simplex antiserum was prepared in rabbits by injection of infected mouse brain suspensions of herpes virus. Adenoviruses were typed by neutralization tests in Hep-2 cell cultures, using antisera prepared in rabbits (8).

[1] From the Section of Preventive Medicine, Department of Medicine; and the Student Health Service, University of Chicago, Chicago, Illinois.

[2] These studies were supported, in part, by a research grant from the Common Cold Foundation and, in part, by the Seymour Coman Fellowship Fund and the Department of Medicine of the University of Chicago.

[3] From Microbiological Associates.

[4] The writers are deeply indebted to Dr. Robert Chanock of the National Institutes of Health who provided them with prototype viruses and antisera for standardization of tests and who also confirmed the complement-fixation titers of the paired sera used for identification of chimpanzee coryza agent.

Serologic tests: All complement-fixation tests were carried out with overnight fixation at 4°C., using the standard procedure for influenza viruses (6). Antigens for influenza viruses were unpurified allantoic fluid harvested from eggs infected forty-eight hours previously with either A2/Jap/305/57 or B/GL/54 virus. Antigen for chimpanzee coryza agent virus was prepared in Hep-2 cell cultures harvested at the time of maximal cytopathogenic effect, frozen and thawed three times, then centrifuged to remove cells and debris. Adenovirus antigen was prepared in a similar way from HeLa cell cultures infected with types 3, 4, and 7 viruses; but infectivity was destroyed by heating at 56°C. for thirty minutes before use. Hemadsorption neutralization tests were carried out by the method described by Chanock and associates by inoculating monkey kidney tissue cultures with mixtures of para-influenza 1 virus and diluted serum which had been incubated *in vitro* for one hour at room temperature (8). After incubation for four days at 37°C., these tissue cultures were washed twice with Hanks basic salt solution and tested for hemadsorption with 0.4 per cent guinea pig cells.

Results

Virus Isolation

Thirty-seven of the 237 throat swab specimens yielded viruses. The number of isolations and the source of specimens are given in table 1. All persons in the groups from the Student Health Service and from the staff and outpatient clinics presented with symptoms of relatively mild upper respiratory infection. Among the Student Health Service population, Group A streptococci were isolated in 12 per cent of the cases. On the other hand, hospitalized patients represented a group with more severe respiratory tract infections. Nineteen per cent of the patients had pneumonias, proved or strongly suspected of being bacterial in origin; 9 per cent were diagnosed as infectious mononucleosis on the basis of physical findings, positive heterophil or abnormal leukocytes in blood smears; and 17 per cent were diagnosed as influenza. The remaining comprised a heterogeneous group which included acute febrile respiratory disease, "viral" pneumonia, and pneumonias of undetermined etiology.

Five adenoviruses were obtained, one type 2, two type 3, one type 7, and one a mixture of types 2 and 5. Two strains of chimpanzee coryza agent virus, five strains of para-influenza 1, and four strains of herpes simplex were isolated. Only one isolation of para-influenza 2 virus was made. The isolation of influenza A2 (Asian) from three specimens and influenza B from 17 specimens occurred during a small outbreak in the late winter of 1959. In this particular outbreak, isolation of influenza B virus in tissue culture proved to be as effective, or possibly even more effective, than inoculation into the amniotic cavity of the chick embryo. Eight specimens which yielded influenza B virus were inoculated into both tissue cultures and chick embryo amnion. Virus was isolated from five of these specimens in both tissue cultures and eggs. Three specimens yielded virus in tissue culture only.

All paired sera collected during this study were tested for antibody to adenovirus, influenza A and B, and chimpanzee coryza agent by complement-fixation tests. Results of these tests are presented in table 2. Paired sera were available from only two of the five persons from whom adenovirus was isolated, and both of these

TABLE 1

VIRUSES ISOLATED FROM 237 THROAT SWABS COLLECTED FROM PERSONS WITH ACUTE RESPIRATORY DISEASE, OCTOBER 1, 1958 TO JUNE 1, 1959

Source of Specimen	Number of Positive Specimens	Virus
Student Health Service, 148 specimens	1	Adenovirus type 3
	1	Adenovirus type 7
	1	Para-influenza 2 (croup associated)
	4	Para-influenza 1 (hemadsorption 2)
	2	Influenza type A2
	9	Influenza type B
	3	Herpes simplex
Hospitalized patients, 58 specimens	1	Adenoviruses types 1 and 5 mixed
	1	Para-influenza 1 (hemadsorption 2)
	1	Chimpanzee coryza agent
	1	Influenza type A2
	2	Influenza type B
	1	Herpes simplex
Outpatients and staff members, 31 specimens	1	Adenovirus type 3
	1	Adenovirus type 2
	1	Chimpanzee coryza agent
	6	Influenza B
Total	37	

TABLE 2

RESULTS OF COMPLEMENT-FIXATION TESTS WITH ADENOVIRUS, INFLUENZA A AND B, AND CHIMPANZEE CORYZA AGENT

Virus Antigen	Persons from Whom Virus Was Isolated			Persons from Whom No Virus Was Isolated	
	Total	Number of Paired Sera	Number with Titer Rises	Total Number of Paired Sera	Number with Titer Rises
Adenoviruses	5	2	2	143	0
Chimpanzee coryza agent	2	1	1	145	1
Influenza A	3	3	3	143	5
Influenza B	17	9	8	143	4

showed a significant rise in titer. No additional infections with this group of viruses were detected serologically among 143 paired sera of persons from whom no virus was isolated. Paired sera were available from only one of the two cases which yielded chimpanzee coryza agent and showed a rise in complement-fixation titer to this virus. One additional infection with this virus was detected by serologic tests. Paired sera from all of the persons whose cultures yielded influenza A showed significant increase in titer. In addition, five cases of influenza were detected by serologic test alone on 143 paired sera. Of the 9 paired sera available from persons infected with influenza B, 8 showed a significant rise in titer and 4 additional cases were detected by serologic tests on 143 paired sera from persons from whom no virus was isolated.

Serologic tests with para-influenza 1 virus were carried out only on paired sera from persons whose specimens yielded this virus. Two of the three persons for whom such sera were available showed a significant rise in antibody titer by hemadsorption neutralization tests. No rise in titer could be demonstrated in the third case.

Although the number of isolations of adenoviruses and para-influenza 1 was small, it is possibly significant that the isolations were made at widely scattered intervals over the entire period of this study. On the other hand, isolations of influenza A2 and influenza B were made chiefly during March and April. The time of occurrence of cases of influenza A and B proved by laboratory tests is given in table 3. Influenza B appeared first; a single case was detected in January and in February. By the first week of March the incidence of influenza B had risen to 9 cases, and the first case of influenza A was detected. From the middle of March through the middle of April, laboratory-proved cases were distributed almost equally between influenza A and influenza B.

Clinical Findings

The clinical findings in adult patients from whom virus was isolated were extremely variable. Of the 2 patients from whom chimpanzee coryza agent was isolated, one showed a clinical picture of infectious mononucleosis with pharyngitis, lymphadenopathy, and hepatosplenomegaly. However, no atypical lymphocytes were seen, and the heterophil was negative. The other patient was ambulatory and had a typical picture of a common cold with cough persisting for three weeks after nasal pharyngeal symptoms had subsided.

Of the 5 patients from whom para-influenza 1 virus was isolated, 3 were afebrile and 2 had mild elevations of temperature. All had coryza and sore throat. The patient from whom para-influenza 2 was isolated had a temperature of 103°F., nasal discharge, injected pharynx, cervical lymphadenopathy, and fine rales scattered throughout the lung fields. The 4 adult patients from whom adenovirus was isolated all showed temperature elevations to 102°F. or greater, and complained of sore throat and cough. Marked injection of the pharynx was noted in all persons; 2 also showed exudate. Wheezing was heard throughout the chest in one patient, and coarse rales were present in a second.

A clinical diagnosis of influenza was made in 14 of the 21 patients with influenza B, upper respira-

TABLE 3

TIME OF OCCURRENCE OF INFLUENZA CASES PROVED BY LABORATORY TESTS

Date, 1959	Number of Cases	
	Influenza A	Influenza B
January 27	0	1
February 24	0	1
March 9–22	1	9
March 23–April 5	3	4
April 6–19	4	4
April 20–27	0	2
Total	8	21

tory tract infection in 6, and viral infection with superimposed bacterial bronchitis in one. Three of the patients were hospitalized. All but one had temperatures ranging between 101° and 103°F. All complained of sore throat, malaise, and nasal congestion. Nine of the 237 specimens examined were obtained from children during the outbreak of influenza. Influenza B was isolated from six of these specimens. All of the 8 patients with influenza A were clinically diagnosed as influenza. Five patients were hospitalized. All had temperatures higher than 102°F. and complained of malaise and cough.

None of the persons from whom herpes simplex virus was isolated showed gross evidence of herpetic infection.

Discussion

By means of laboratory procedures involving either isolation of virus from throat swabs or serologic tests on paired sera, viruses have been implicated in 43 of the 237 cases studied. This excludes isolation of herpes simplex, which is frequently associated with "colds" but represents recrudescence of a latent infection (9). An outbreak of influenza, shown to involve both influenza A2 and influenza B viruses, accounted for about 12 per cent of the total respiratory disease. Occurrence of both types of influenza virus in a single outbreak is not usual, but has been reported several times in the last twenty-five years (10). None of the cases in the present study represented concurrent infection with both viruses.

About 6 per cent of the cases involved infection with either adenoviruses, para-influenza 1 and 2, or chimpanzee coryza agent. The low incidence of adenovirus infection in adults confirms previous reports which indicated that these viruses were not common except among military recruit populations (11). Although para-influenza 1 has been shown to infect human volunteers (12), there is little information concerning its association with respiratory disease in adults. Meenan and co-workers (13) isolated this virus from one adult with acute respiratory disease, and recently Dick and Mogabgab (14) reported isolation of 11 strains during the fall and early winter of 1958–1959, all from cases of mild upper respiratory infection in adults. Isolation of para-influenza 2 or chimpanzee coryza agent from adults has not previously been reported.

At the present time, all of the available information points to a multiplicity of viruses involved in infections of the respiratory tract of man. However, further study is necessary to determine the relative importance of some of these agents and to delineate more precisely outbreaks caused by them.

Summary

Two hundred and thirty-seven patients with respiratory disease were studied in an effort to make an etiologic diagnosis. In only 43 cases were known viruses implicated. Influenza A and B accounted for 29 illnesses, or 12 per cent of the total. Adenovirus, para-influenza 1 and 2, and chimpanzee coryza agent appeared to be related to illness in 14 cases, or 6 per cent of the total. The etiology of the vast majority of respiratory disease remains undefined.

Sumario

Estudio de los Virus Inculpados en las Enfermedades Agudas del Aparato Respiratorio en Chicago de octubre de 1958 a junio de 1959

Con mira a hacer un diagnóstico etiológico, se estudió a 237 enfermos que tenían afecciones del aparato respiratorio. Sólo en 43 casos figuraron virus conocidos. La influenza A y la B intervinieron 29 veces, o sea 12 por ciento del total. El adenovirus, la parainfluenza 1 y la 2 y el factor del coriza del chimpancé parecían guardar relación con la enfermedad presente en 14 casos, o sea 6 por ciento del total. La etiología de la inmensa mayoría de los casos de enfermedad respiratoria permanece indefinida.

Resume

Une étude des virus impliqués dans la maladie respiratoire aiguë de Chicago, d'Octobre 1958 à Juin 1959

Dans un effort pour faire un diagnostic étiologique, les auteurs ont étudié deux cent trente sept malades ayant une maladie respiratoire. Dans seulement 43 cas, des virus connus furent impliqués. L'influenza A et B furent responsables de 29 maladies, soit 12% du total. L'adenovirus, le parainfluenza 1 et 2 et l'agent du coryza du chimpanzé semblèrent être en relation avec la maladie dans 14 cas, soit 6% du total. L'étiologie de la vaste majorité de la maladie respiratoire reste indéfinie.

Acknowledgment

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LEUKOCYTOTOXIC COMPONENT OF PINE POLLEN[1]

Effect on Peripheral Blood Leukocytes of Normal and Tuberculin-hypersensitive Guinea Pigs

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INTRODUCTION

Epidemiologic studies of cases of sarcoidosis seen in Veterans Administration hospitals revealed an interesting geographic distribution of the disease in the United States which correlated well with pine forest distribution (1). In recent studies in this laboratory (2), pine pollen was found to have acid-fast staining properties and to contain phospholipid, chloroform-soluble waxes, alcohol-ether–soluble lipids, and firmly bound lipids similar to those described for the tubercle bacillus. Moreover, pine pollen was found capable of producing localized epithelioid granulomas in guinea pigs. It was the purpose of the present study to determine whether the lipids of pine pollen possessed any toxicity for peripheral blood leukocytes of normal guinea pigs and, if so, how this activity compared with that of similar fractions from tubercle bacilli. In addition, it was thought of interest to conduct comparative studies on the effect of the material on the blood leukocytes of normal and tuberculin-hypersensitive guinea pigs.

MATERIALS AND METHODS

Fractionation of pine pollen and tubercle bacilli: Loblolly pine pollen[2] was used throughout this study. The pollen had been collected in April, 1958, in Macon, Georgia, and stored at 5°C. Six-week-old cultures of virulent tubercle bacilli (H37Rv[3]) grown on a modified Proskauer and Beck liquid medium (3) were autoclaved for forty-five minutes at 121°C., filtered on a Buchner funnel through Whatman No. 1 paper, and washed with distilled water until the washings were clear. The bacillary mass was then transferred to a Petri dish, dried for one hour at 110°–130°C., pulverized in a mortar, and weighed.

Suitable samples of pine pollen and dried tubercle bacilli were extracted for four days at room temperature, with frequent shaking, in a 2:1 mixture of methanol and ethyl ether. The suspensions were then centrifuged, and the sediment was extracted twice more with methanol-ether. The pooled methanol-ether extracts were passed through a Seitz filter, concentrated to 5 ml. on a 60°C. water bath under a stream of nitrogen, and extracted three times with petroleum ether. The pooled petroleum ether extracts were then concentrated to small volume under nitrogen, and the phospholipids were precipitated by the addition of an excess of cold (0°C.) acetone. After removal of the phospholipids by centrifugation, the acetone-soluble fraction was dried to constant weight on a 60°C. water bath under a stream of nitrogen, re-dissolved in 3 ml. of acetone, and stored at 5°C.

For comparative purposes a purified Wax C was also prepared from the tubercle bacilli by the following technique (4): The bacillary mass, after it had been extracted thoroughly with methanol-ether, was further extracted with chloroform overnight at room temperature. The suspension was then filtered on a Buchner funnel through Whatman No. 1 filter paper, and the cell mass on the filter paper was washed thoroughly with chloroform. The washings were pooled with the original filtrate. The chloroform extract was then passed through a Seitz filter, dried to constant weight on a 60°C. water bath under a stream of nitrogen, dissolved in a small volume of ethyl ether, and the "purified wax" was precipitated by adding an excess of methanol in the cold (5°C.). The purified wax was recovered by centrifugation, washed thoroughly with cold methanol, and dissolved in 4 ml. of benzene to which 1.5 parts of acetone were added slowly with stirring. The gummy precipitate (wax D) was discarded. Wax C was precipitated from the clear supernate by the addition of an excess of methanol in the cold (5°C.), recovered by centrifugation, washed thoroughly with cold methanol, dried to constant weight on a 50°C. water bath under a stream of nitrogen, redissolved in 6 ml. of chloroform, and stored at 5°C.

Twenty milligrams of purified cord factor were obtained through the courtesy of Dr. Hubert Bloch, Department of Microbiology, University of Pittsburgh.

For use in certain of the studies a crude protein extract of loblolly pine pollen was isolated as follows: Five grams of pollen were prepared as a thin paste in 0.5 per cent sodium chloride (adjusted to pH 7.8 with sodium bicarbonate) and

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[1] From the Tuberculosis Research Laboratory, Veterans Administration Hospital, Washington, D. C.

[2] Generously supplied by the Forest Products Laboratory, United States Department of Agriculture, Madison, Wisconsin.

[3] Culture originally supplied by Dr. William Steenken, Jr., Saranac Lake, New York.

ground for eight hours in a ball mill.[4] The suspension was then made up to 100 ml. with the alkaline saline, centrifuged, and the supernate was passed through Whatman No. 44 filter paper on a Buchner funnel. The filtrate, after readjusting the volume to 100 ml. with saline, was brought to pH 4.1 by the gradual addition of a small volume of glacial acetic acid. The rather voluminous precipitate was recovered by centrifugation, redissolved in alkaline saline, purified by reprecipitation at pH 4.1, dissolved in 50 ml. of saline, and the pH was adjusted to 7.0 with N/10 sodium hydroxide. The material was dialyzed for two days in the cold (5°C.) against frequent changes of the alkaline (pH 7.8) saline, and the volume was adjusted to 100 ml. Traces of insoluble material were removed by centrifugation, and the supernatant fluid was stored at 5°C. Suitable aliquots were used for the determination of total nitrogen by nesslerization. Protein nitrogen was determined on phosphotungstic-hydrochloric acid precipitates. Replicate analyses revealed the presence of 4.3 mg. per 100 ml. total nitrogen, 77 per cent of which was associated with protein.

Sensitization of guinea pigs: Male, albino guinea pigs (400–500 gm.) were used throughout the study. They were maintained on a diet of commercial pellets and fresh cabbage. Animals were sensitized to tuberculin by a single intramuscular injection of 1.0 ml. of sterile paraffin oil containing 5 mg. of heat-killed human tubercle bacilli (H37Rv). Intradermal skin tests were done with 5 γ of PPD (Sharp and Dohme).

Measurement of leukocyte injury: Damage to leukocytes was measured by a technique based on the release of an intracellular, lysozyme-like enzyme from injured cells (5). For each guinea-pig blood to be tested, four siliconized serologic tubes were prepared. To two tubes 0.1 ml. of fraction to be tested was added; to two tubes 0.1 ml. of diluent was added. To each of the four tubes was then added 0.6 ml. of freshly collected guinea-pig whole blood (heparinized). All animals were made to fast overnight prior to bleeding. The tubes were sealed with silicone stoppers, rotated at 37°C. on a roller drum for two hours, and centrifuged. Assays for lysozyme-like enzyme were performed on 0.1-ml. aliquots of each supernatant fluid (5). An index of cellular damage was calculated by subtracting the mean per cent lysis of substrate by diluent tubes from that obtained with experimental tubes. In some cases a relative index of damage was calculated as:

$$\frac{\text{index of damage, experimental cells}}{\text{index of damage, normal cells}}$$

Since the lipid fractions were insoluble in water, some difficulty was encountered in obtaining stable suspensions for use in the tests. After a considerable amount of investigation with various manipulations, the following technique was found to be suitable: The acetone-soluble fraction from pine pollen and the acetone-soluble fraction, wax C, and cord factor from tubercle bacilli were dried under nitrogen and dissolved in a small volume of acetone. While the acetone solutions were maintained on a 60°C. water bath, 0.9 per cent sodium chloride was added slowly, drop by drop, until the desired concentration of lipid was achieved (usually 5 mg. per ml.) and the final concentration of acetone was 10 per cent. This resulted in suspensions which, although tending to settle out on standing, were readily resuspended by gentle shaking on a 60°–70°C. water bath. Preliminary trials indicated, however, that the amount of acetone present in 0.1-ml. portions of such suspensions interfered in the test, presumably by blocking the damage to leukocytes of potentially cytotoxic materials. Further investigation revealed that the acetone could be removed simply by placing the tubes, both experimental and control, in a 60°–70°C. water bath for thirty minutes under an exhaust fan. After the tubes had cooled, whole blood was added and tests were conducted in the usual manner.

Whenever blood from tuberculin-hypersensitive guinea pigs was studied, the cytotoxicity of PPD was also measured, as well as that of a polysaccharide I[5] component of the unheated culture filtrate of virulent tubercle bacilli. Commercial PPD tablets (Sharp and Dohme) and polysaccharide I were diluted in physiologic saline. Experimental tubes contained 0.15 ml. (7.5 γ) of PPD or 0.15 ml. (17.0 γ) of polysaccharide. Control tubes received an equal volume of saline.

In order to determine whether leukocytic susceptibility to the various test materials correlated with qualitative or quantitative changes in the peripheral blood picture, total and differential leukocyte counts were done on representative samples.

Statistical analysis: Correlation coefficients and "*t*" tests for the significance of difference between means were calculated according to methods of Croxton (8).

Results

In a series of preliminary studies with the acetone-soluble fraction of tubercle bacilli, a concentration of 0.4 to 0.8 mg. per tube was found to cause consistent, significant damage to normal guinea-pig leukocytes, as determined by the lysozyme-assay method. A concentration of 0.5 mg. per tube was therefore chosen for use

[4] The writers are indebted to Mr. Paul C. Hudgins for suggesting this technique.

[5] This material, isolated in this laboratory (6) from unheated culture filtrates of the H37Rv strain by a technique modified from that of Seibert (7), retains her terminology. Preliminary studies have suggested, however, that it may be a glycoprotein.

TABLE 1

EFFECT OF VARIOUS FRACTIONS OF PINE POLLEN AND OF VIRULENT TUBERCLE BACILLI ON LEUKOCYTES OF NORMAL GUINEA PIGS

Material*	Mean Index of Cellular Damage
Pine pollen	
(*a*) Acetone-soluble fraction (20)	8.0
(*b*) Protein (20)	0.1
Tubercle bacilli (H37Rv)	
(*a*) Acetone-soluble fraction (16)	6.0
(*b*) Wax C (12)	1.9
Cord factor (8)	3.9

* Figure in parenthesis indicates number of animals.

in comparative studies on the effect of the various materials on the leukocytes of normal guinea pigs.

Because some of the materials were in rather short supply not all of them could be tested against all of the animals. The results are presented in table 1. For comparative purposes, it seems pertinent to state that, from figures accumulated over the years in this laboratory, the mean index of cellular damage of PPD and polysaccharide I in normal guinea pigs is 0.4 and 0.5, respectively. Exhaustive preliminary trials measuring the effect of PPD on leukocytes from tuberculin-sensitive guinea pigs indicated that an index of 2.0 or more usually represents significant cellular damage. On this basis and at the concentration level used, the acetone-soluble fraction from pine pollen, the acetone-soluble fraction from tubercle bacilli, and the cord factor all damaged normal guinea-pig leukocytes to a significant degree. It is of particular interest that, with the 16 animals whose blood was tested with both substances, there was a highly significant ($P < 0.001$) coefficient of linear correlation (0.77) between the indices of damage by the pine pollen acetone-soluble and H37Rv acetone-soluble fractions.

The results of this study suggested that it might be of interest to determine whether sensitization to tuberculin alters the susceptibility of guinea pig leukocytes to the acetone-soluble fraction of pine pollen and, if so, whether it correlates with altered susceptibility to PPD and/or the polysaccharide I fraction of tubercle bacilli (5, 6).

For this purpose, 16 pigs were injected with heat-killed tubercle bacilli in paraffin oil. The animals were bled in groups of 4 at five, twelve, nineteen, and twenty-six days after injection. The susceptibility of blood leukocytes to PPD, polysaccharide I, pine pollen acetone-soluble fraction and, for comparative purposes, to the pine pollen protein extract was measured by the technique described previously.

The results are shown in table 2. It may be seen that at nineteen and twenty-six days after injection with heat-killed tubercle bacilli there was a significant increase in susceptibility of guinea-pig leukocytes to PPD, polysaccharide I, and the acetone-soluble fraction of pine pollen. Total and differential leukocyte counts revealed that these same animals had mean absolute neutrophil and lymphocyte counts higher ($P < 0.05$) than normal. A word of explanation should perhaps be given for the relatively low (1.6 and 4.8) mean indices of damage produced by the pine pollen acetone-soluble fraction in this study. Although this material was prepared by the same technique, for some as yet unexplained reason it was less cytotoxic at the 0.5 mg. level than the lot used in obtaining the results presented in table 1. In a group of 23 normal guinea pigs it gave a mean index of cellular damage of 2.2.

A rather unexpected finding in the study just described was the fact that, while injection with heat-killed tubercle bacilli appeared to increase susceptibility of guinea pig leukocytes to the acetone-soluble fraction of pine pollen, there was no alteration in susceptibility to the protein extract of pollen. The protein extract used, although it had been stored at 5°C., was more than five months old. Since some deterioration

TABLE 2

SUSCEPTIBILITY OF GUINEA-PIG LEUKOCYTES TO PPD, POLYSACCHARIDE I, PINE POLLEN ACETONE-SOLUBLE FRACTION, AND PINE POLLEN PROTEIN FRACTION AFTER INJECTION WITH HEAT-KILLED TUBERCLE BACILLI

Days post Injection	Mean Index of Cellular Damage			
	PPD	Polysaccharide I	Pine Pollen Acetone-soluble Fraction	Pine Pollen Protein Fraction
5 and 12	0.4	0.6	1.6	1.7
19 and 26	3.8*	4.1*	4.8*	1.1

* Statistically significant, $P < 0.025$.

TABLE 3

SUSCEPTIBILITY OF GUINEA-PIG LEUKOCYTES TO PPD, POLYSACCHARIDE I, AND PINE POLLEN PROTEIN FRACTION AFTER INJECTION WITH HEAT-KILLED TUBERCLE BACILLI

Guinea Pigs	Mean Index of Cellular Damage		
	PPD	Polysaccharide I	Pine Pollen Protein Fraction
Normal	1.2	0.0	1.8
Experimental	8.4	7.3	1.7

could conceivably have occurred, further studies were made using freshly prepared material.

Five normal guinea pigs and 10 guinea pigs which had been injected with heat-killed tubercle bacilli three to five months previously were bled in the usual fashion and the leukocytotoxic effect of PPD, polysaccharide I, and the freshly prepared protein fraction of pine pollen was determined.

The results are presented in table 3. As in the preceding study, peripheral blood leukocytes from animals injected with heat-killed tubercle bacilli demonstrated an increased susceptibility to PPD and polysaccharide I. Again, however, there was no alteration in cellular susceptibility to the pine pollen protein fraction.

The findings presented in table 2 suggested that, in animals injected with heat-killed tubercle bacilli, blood leukocytes showing an increased susceptibility to the acetone-soluble fraction of pine pollen also showed an increased susceptibility to PPD and polysaccharide I. Since previous investigations in this laboratory (9) revealed that leukocytic susceptibility to PPD and polysaccharide I do not necessarily always correlate in tuberculin-sensitive guinea pigs, further studies were done.

Sixteen guinea pigs were injected with heat-killed tubercle bacilli. Four months later, when intradermal skin tests on representative animals produced reactions averaging 10 mm. of induration at forty-eight hours, the animals were bled and leukocytic susceptibility to PPD, polysaccharide I, and the acetone-soluble fraction of pine pollen was measured.

The results are presented in table 4. Blood leukocytes from only 7 of the 16 animals proved to be highly susceptible to the pine pollen acetone-soluble fraction. Cells from these same animals were also significantly susceptible to PPD but not to the polysaccharide I. The lack of statistical significance for the mean index of damage with polysaccharide I (4.0) can probably be attributed to the poor correlation between the indices for PPD and polysaccharide I in this group of animals. Further support for the close correlation between susceptibility to PPD and to the acetone-soluble pine pollen fraction was found in calculations of coefficients of correlation. When results from all 16 animals were used, the coefficient of linear correlation (0.80) between indices for PPD and the pine pollen acetone-soluble fraction was better than that (0.56) between polysaccharide I and pollen. It is pertinent to point out that the mean absolute number of neutrophils and lymphocytes did not differ significantly in the two groups (*A* and *B*, table 4).

Further support for the suggestion that susceptibility to pine pollen acetone-soluble frac-

TABLE 4

SUSCEPTIBILITY OF GUINEA-PIG LEUKOCYTES TO PPD, POLYSACCHARIDE I, AND PINE POLLEN ACETONE-SOLUBLE FRACTION AFTER INJECTION WITH HEAT-KILLED TUBERCLE BACILLI

Group*	Mean Index of Cellular Damage		
	Pine Pollen Acetone-soluble Fraction	PPD	Polysaccharide I
A (9)	2.3	1.9	0.5
B (7)	10.6†	6.8†	4.0

* Grouped according to low (*A*) and high (*B*) mean indices of damage with pine pollen acetone-soluble fraction. Numbers in parentheses indicate number of animals.

† Statistically significant, $P < 0.025$.

TABLE 5

SUSCEPTIBILITY OF GUINEA-PIG LEUKOCYTES TO PPD, POLYSACCHARIDE I, AND PINE POLLEN ACETONE-SOLUBLE FRACTION AFTER INJECTION WITH HEAT-KILLED TUBERCLE BACILLI

Material	Relative Index of Cellular Damage
PPD	1.4
Pine pollen acetone-soluble fraction	0.7
Polysaccharide I	9.2*

* Statistically significant, $P < 0.001$.

tion correlates better with susceptibility to PPD than to polysaccharide I came from a final study.

A group of 13 guinea pigs was bled two to three weeks after injection with heat-killed tubercle bacilli, and whole blood samples were used to measure leukocytic susceptibility to PPD, polysaccharide I, and the acetone-soluble fraction of pine pollen in the usual manner.

Leukocytes from these animals showed no increase in susceptibility to either PPD or the pine pollen acetone-soluble fraction and yet were extremely susceptible to polysaccharide I (table 5).

Discussion

Nasta and associates (10) extracted tubercle bacilli successively with acetone, ether, methanol, and ethanol and reported that only the acetone-soluble fraction was cytotoxic for normal guinea-pig leukocytes *in vitro*. An estimate of cellular damage was based on calculation of the percentage of viable cells, through the use of vital stains, after two hours at 37°C. The results of the present study confirm the cytotoxic activity of the acetone-soluble fraction of tubercle bacilli by a technique based on the release of an intracellular lysozyme-like enzyme from damaged cells. Kerby (11) used a similar method to study the damaging effect of bacterial derivatives on human leukocytes. The present results suggest that the method might be applicable to the study of other reportedly toxic derivatives of tubercle bacilli and, in addition, show that an acetone-soluble fraction of loblolly pine pollen appears to be as toxic for normal guinea-pig leukocytes as a comparable fraction from virulent tubercle bacilli. It is realized that acetone-soluble leukocytotoxic substances probably do not reside exclusively in pine pollen and tubercle bacilli but might be characteristic of a variety of biologic materials.

Certain precautions must be observed in measuring leukocyte injury by a technique based on the determination of enzyme released from damaged cells. If, as in the present study, the effects of foreign biologic materials are being examined for leukocytotoxic properties, it is important to know whether the test substance might interfere somehow in the enzyme assay system and thus lead to false interpretation. All of the substances used here were studied exhaustively in a leukocyte-free system and were found to have no measurable effect on enzyme substrate in the usual dosage employed.

Insofar as interpretation of results is concerned, the writers realize that 0.5 mg. of test substance per 0.6 ml. of whole blood represents a rather large dosage when it is considered that this weight of acetone-soluble extractives, for example, would represent approximately 12 mg. (dry weight) of intact tubercle bacilli or 30 mg. of pine pollen based on yields obtained by the technique of extraction used. It should be borne in mind, however, that the *in vitro* system used is at best a highly artificial one. As mentioned earlier, the test substances were in the form of rather unstable suspensions. Examination of the reaction tubes at the completion of each study revealed that much of the material had coated the sides of the tubes, and it seems reasonable to suspect that only a small quantity actually came in contact with leukocytes. In addition, it should be mentioned that, with increasing availability and purity of the test materials, a more thorough investigation of optimal conditions required to demonstrate cell damage might reveal that much smaller quantities are actually required.

The finding that extractives of tubercle bacilli are toxic for normal leukocytes was not particularly surprising. The work of Nasta and associates has already been mentioned. In addition, the cord factor of Bloch (12), the lipopolysaccharide of Choucroun (13), and phthienoic acid (14) have all been shown to inhibit migration of normal leukocytes in tissue culture. The role that these and other toxic components of tubercle bacilli (15) play in the pathogenesis of the disease has not yet been clearly defined.

In the tuberculin-sensitive host, the lysis of peripheral blood leukocytes *in vitro* by tuberculin appears to be an antigen-antibody reaction mediated by a factor in the globulin fraction of plasma (16) and requiring complement (17). It was of some interest to find that blood leukocytes of guinea pigs injected with heat-killed tubercle bacilli show a susceptibility to the acetone-soluble fraction of pine pollen which seems to correlate well with susceptibility to PPD. In contrast, there was rather poor correlation with the polysaccharide I fraction isolated from unheated culture filtrates of virulent tubercle bacilli. As mentioned previously, studies in this laboratory have revealed that, in tuber-

culin-sensitive guinea pigs, leukocytic susceptibility to PPD and polysaccharide I do not always correlate (9). Susceptibility to polysaccharide I, once established, appears to persist for some time, while susceptibility to PPD waxes and wanes. It is tempting to speculate that there is present in the acetone-soluble fraction of loblolly pine pollen a constituent related to PPD. If this is so, then it would have to be concluded that the cross-reacting component is present in the acetone-soluble fraction of pollen but not in that portion of saline-extractable material insoluble at pH 4.1. The latter material, in two separate experiments, appeared to have no effect on leukocytes of tuberculin-sensitive animals. Comparative studies with cord factor, wax C, and/or the acetone-soluble fraction of tubercle bacilli might eventually shed some light on the problem. Unfortunately, sufficient quantities of these materials were not available to include them in the entire series of experiments.

It has been observed repeatedly that the tuberculous host reacts hyperergically to a variety of apparently nontuberculous stimuli. Suter and associates (18), for example, have reported that mice infected with BCG become highly sensitive to lipopolysaccharide derived from gram-negative bacteria. The fact that cord factor induced a similar state of hyperreactivity to endotoxin seemed to indicate that the phenomenon was not related to delayed hypersensitivity, since cord factor does not induce sensitivity of this type. By the same token, it must be admitted that the increased susceptibility of PPD-sensitive leukocytes to an acetone-soluble extract of pine pollen may be only an expression of a general vulnerability of sensitized cells to injury by certain nonspecific substances, quite independent of specific tuberculin reactivity.

Summary

An acetone-soluble fraction has been extracted from loblolly pine pollen which appears to be as toxic for normal guinea pig leukocytes *in vitro* as a comparable fraction from virulent tubercle bacilli. Blood leukocytes from guinea pigs injected with heat-killed tubercle bacilli show a susceptibility to the acetone-soluble fraction of pine pollen which seems to correlate well with susceptibility to PPD but not with susceptibility to a polysaccharide I (glycoprotein) fraction isolated from unheated culture filtrates of virulent tubercle bacilli.

Sumario

Componente Leucocitotóxico del Polen de Pino. Efecto sobre los Leucocitos de la Sangre Periférica de los Cobayos Normales y los Hipersensibles a la Tuberculina

Del polen del pino *taeda* se ha extraído una fracción acetonosoluble que parece ser tan tóxica para los leucocitos del cobayo normal *in vitro* como una fracción comparable de bacilos tuberculosos virulentos. Los leucocitos sanguíneos de los cobayos a los que se inyectaran bacilos tuberculosos matados al calor revelan una susceptibilidad a la fracción acetonosoluble del polen que parece correlacionarse con la susceptibilidad al PPD (DPP), pero no con la susceptibilidad a una fracción del polisacárido I (glucoproteína) aislada de los filtrados culturales no calentados de bacilos tuberculosos virulentos.

Resume

Composé leucocytotoxique du pollen du pin: son effet sur les leucocytes du sang périphérique de cobayes normaux et de cobayes hypersensibles à la tuberculine

Une fraction soluble dans l'acétone a été extraite du pollen du pin "loblolly", qui semble, *in vitro*, être aussi toxique pour les leucocytes du cobaye normal qu'une fraction comparable de bacilles tuberculeux virulents. Les leucocytes du sang de cobaye à qui on a injecté des bacilles tuberculeux tués par la chaleur montre une sensibilité à la fraction de pollen de pin soluble dans l'acétone, laquelle semble bien être en relation avec la sensibilité à PPD, mais pas avec la sensibilité à une fraction de polysaccharide I (glucoprotéine) isolée à partir de filtrats de cultures non chauffées de bacilles tuberculeux virulents.

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AN EVALUATION OF CHEMOTHERAPY IN PULMONARY TUBERCULOSIS[1]

High Doses of Isoniazid plus PAS versus Low Doses of Isoniazid plus PAS

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Introduction

There have been numerous reports in the literature during the past few years pertaining to the clinical results obtained when high doses of isoniazid (10 to 20 mg. per kg. daily) are used in the treatment of pulmonary tuberculosis. In studies of this nature, the eventual knowledge desired is: are treatment programs which include high doses of isoniazid therapeutically superior to programs which include low or conventional doses (300 mg. daily) of isoniazid. Reports have been published which suggest that high doses of isoniazid may enhance the treatment results in tuberculosis, particularly in those patients who rapidly inactivate a major portion of a given dose of isoniazid after it has been absorbed (1-3). Within the past year, there have been investigations which tend to reverse some of these original concepts (4, 5). These aforementioned studies now suggest that there may not be any significant difference in the treatment results in tuberculous patients when high-dosage isoniazid therapy is compared with low-dosage isoniazid therapy. The following study was designed to determine whether there is any detectable difference in the results of treatment in two similar groups of patients when high doses of isoniazid plus aminosalicylic acid are used in contrast with low doses of isoniazid plus aminosalicylic acid. The high-dose isoniazid regimen also included 100 mg. of pyridoxine each day.

Materials and Methods

The criteria for selection of all of the cases were as follows: (*1*) all patients were previously untreated or had received less than two months of therapy (in no case, streptomycin) immediately prior to admission; (*2*) cultures positive for *M. tuberculosis* were obtained from sputum or gastric specimens; (*3*) only moderately and far advanced cases of pulmonary tuberculosis were selected; and (*4*) the patients must have been under observation in the hospital for more than three months.

Between January and December, 1959, 91 patients who met these criteria were selected on a consecutive admission basis and treated with low or conventional doses of isoniazid, 300 mg. daily, plus aminosalicylic acid (PAS), 12 gm. daily. The 91 patients were then compared with 113 previously selected patients (6) who had been treated with high doses of isoniazid (10-20 mg. per kg. daily), PAS (12 gm. daily), and pyridoxine (100 mg. daily). The type of case material was similar in both groups. In table 1 the classification of cases selected for the high and low isoniazid treatment groups is outlined. The percentage of moderately and far advanced cases in each treatment group is similar. There are slightly more cavitary cases and more multiple cavitary cases in the low isoniazid–PAS—treatment group.

The concentrations of free isoniazid in the serum of 190 of the 204 patients were determined six hours after one-third of the total daily doses of isoniazid and PAS had been administered.[3] In the low-isoniazid group, this determination was made after a test dose of 5 mg. of isoniazid per kg. had been administered.

In table 2 it may be seen that approximately twice as many patients in the low isoniazid–PAS group, as compared with the high isoniazid–PAS group, had serum concentrations of free isoniazid which were below 0.6 γ per ml.

Planigrams were taken of all patients recorded as having closed cavities. When a cavity disappeared from view on conventional posterior-anterior and lordotic chest roentgenograms, planigrams were made. If at least two of three physicians failed to see evidence of cavitation, the case was recorded as a closed cavitary case. Moderate to marked roentgenographic improvement refers to greater than 50 per cent resolution of abnormal shadows (excluding cavities) in comparison to the extent of disease on admission to the hospital. As with the cavitary cases, a three-man board was used in making determinations of roentgenographic improvement.

All 204 cases in both treatment groups were treated on an ambulatory basis from the time of admission. No rest periods were recommended,

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[3] The bioassay technique used was taken from Laboratory Methods for Tuberculosis Bacteriology, U. S. Army Research Development Unit, Fitzsimons Army Hospital, Denver, Colorado, revised edition (1957).

TABLE 1

CLASSIFICATION OF CASES

	High Isoniazid-PAS Group	Low Isoniazid-PAS Group
Total number of cases	113	91
Moderately advanced cases	79.6%	82.4%
Far advanced cases	20.4%	17.6%
Total number of cavitary cases	76.1%	85.7%
Single cavities	50.5%	39.5%
Multiple cavities	25.6%	46.2%

TABLE 2

CONCENTRATIONS OF FREE ISONIAZID IN THE SERUM

	Number of Cases Tested	Per Cent with Concentrations below 0.6 γ per Ml.
High* isoniazid-PAS group	100	22.0
Low† isoniazid-PAS group	90	45.6

* Serum collected 6 hours after ⅓ total daily dose of isoniazid.

† Serum collected 6 hours after a 5-mg. per kg. test dose of isoniazid.

and the patients were allowed to walk through the hospital corridors to medical and occupational therapy clinics and to utilize outdoor areas. After cultures became negative for tubercle bacilli and significant clinical improvement occurred, the patients were given limited calisthenics and job assignments equivalent to a forty-hour work week. After six to fifteen months of hospitalization, the patients were discharged with instructions to continue chemotherapy for an additional six to twelve months.

In neither group was any pulmonary collapse or excisional therapy employed during the period of the evaluation of the antimicrobial therapies.

RESULTS

In tables 3 through 9, the incidence of cultures negative for *M. tuberculosis* after three and five to six months has been tabulated. More than 90 per cent of the patients in both treatment groups had cultures negative for *M. tuberculosis* after three months of treatment (table 3). After five to six months, 97.4 to 99 per cent of the 204 patients had negative cultures.

The incidence of negative cultures in moderately advanced cases after three and five to six months was similar to the results recorded for all of the cases (table 4). Cultures were negative for tubercle bacilli in 96.8 to 99 per cent of the patients after five to six months. A much smaller number of far advanced cases was studied, and all of the patients had negative cultures after five to six months (table 5).

In tables 6 and 7 it may be seen that slightly more noncavitary than cavitary cases were associated with negative cultures after three months, but there was little difference between the two treatment groups. After five to six months, 3 per cent or less of all of the patients were still discharging tubercle bacilli and all of the noncavitary cases in both treatment groups had attained noninfectious status.

There was a greater difference in the incidence

TABLE 3

BACTERIOLOGIC STUDIES

(All Cases)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Negative Cultures	Per Cent with Negative Cultures	Number of Cases
3	113	94	90.1	91
5–6	109	99	97.4	76

TABLE 4

BACTERIOLOGIC STUDIES

(Moderately Advanced Cases)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Negative Cultures	Per Cent with Negative Cultures	Number of Cases
3	90	96.6	90.6	75
5–6	88	99.0	96.8	63

TABLE 5

BACTERIOLOGIC STUDIES

(Far Advanced Cases)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Negative Cultures	Per Cent with Negative Cultures	Number of Cases
3	23	82.5	87.5	16
5–6	21	100.0	100.0	13

TABLE 6

BACTERIOLOGIC STUDIES

(Noncavitary Cases)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Negative Cultures	Per Cent with Negative Cultures	Number of Cases
3	27	92.5	100	13
5–6	27	100.0	100	11

TABLE 7

BACTERIOLOGIC STUDIES

(Cavitary Cases)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Negative Cultures	Per Cent with Negative Cultures	Number of Cases
3	87	87.2	88.5	78
5–6	82	98.8	97.0	65

TABLE 8

BACTERIOLOGIC STUDIES

(Single Cavities)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Negative Cultures	Per Cent with Negative Cultures	Number of Cases
3	57	94.7	83.4	36
5–6	54	98.2	93.3	30

TABLE 9

BACTERIOLOGIC STUDIES

(Multiple Cavities)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Negative Cultures	Per Cent with Negative Cultures	Number of Cases
3	29	79.3	93	42
5–6	28	100.0	100	35

of cultures negative for tubercle bacilli after three months of treatment when the cavitary cases were compared with respect to single and multiple cavities (tables 8 and 9). After three months, cultures for tubercle bacilli were negative in 10 to 15 per cent more of the single cavitary cases. After five to six months of treatment, all of the multiple cavitary cases had negative cultures, and 93.3 and 98.2 per cent, respectively, of the single cavities in the low- and high-isoniazid treatment groups were associated with negative cultures.

Cavity closures in the high-isoniazid–PAS group versus the low-isoniazid–PAS group are compared in tables 10 through 14. In all of the cases (table 10), 7.1 per cent more cavities were

TABLE 10

CAVITY CLOSURE

(All Cavitary Cases)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Closed Cavities	Per Cent with Closed Cavities	Number of Cases
3	86	17.5	1.3	78
5–6	82	54.8	47.7	65

TABLE 11

CAVITY CLOSURE

(Single Cavities)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Closed Cavities	Per Cent with Closed Cavities	Number of Cases
3	57	26.3	22.2	36
5–6	54	63.0	56.6	30

TABLE 12

CAVITY CLOSURE

(Multiple Cavities)

	High Isoniazid-PAS Group		Low Isoniazid-PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Closed Cavities	Per Cent with Closed Cavities	Number of Cases
3	29	3.4	4.8	42
5–6	28	42.8	40.0	35

TABLE 13

CAVITY CLOSURE

(Cases with Serum Isoniazid Concentrations below 0.6 γ per Ml.)

	High Isoniazid–PAS Group		Low Isoniazid–PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Closed Cavities	Per Cent with Closed Cavities	Number of Cases
3	28	14.3	19.5	41
5–6	28	42.8	55.9	34

TABLE 14

CAVITY CLOSURE

(Cases with Serum Isoniazid Concentrations above 0.6 γ per Ml.)

	High Isoniazid–PAS Group		Low Isoniazid–PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent with Closed Cavities	Per Cent with Closed Cavities	Number of Cases
3	51	13.7	5.6	36
5–6	51	49.0	38.7	31

closed in the high-isoniazid group after five to six months, and the rates of cavity closure were 47.7 and 54.8 per cent, respectively, in the low- and high-isoniazid–PAS groups. (As indicated below, however, these differences were not demonstrably significant on statistical analysis.)

In tables 11 and 12, single-cavity versus multiple-cavity closures are tabulated. Very few multiple cavities closed after three months, and 16–20 per cent more single cavities in both treatment groups were closed after five to six months. When the high- and low-isoniazid groups were compared at five to six months, only 2.8 and 6.4 per cent more cavities in the multiple and single cavitary groups, respectively, closed in the high-isoniazid group. The chi-square method was applied to the data in tables 10, 11, and 12, and indicated that the difference in results of cavity closure between the two treatment groups was not statistically significant.

In tables 13 and 14, the incidence of cavity closure is tabulated with relation to the concentration of free isoniazid in the serum six hours after a given dose. A serum concentration of 0.6γ per ml. was chosen as the division between slow and rapid inactivators of isoniazid. A biologic method of assay was used, and free isoniazid concentrations were reported in γ per ml. as follows: 0.15–0.3, 0.3–0.6, 0.6–1.2, 1.2–2.4, 2.4–4.8, and 4.8–9.6. Since this method is a serial tube dilution technique, a concentration of 0.6γ actually represents a range of 0.6 to 1.2γ per ml. of free isoniazid. In table 13, those cases which appeared to represent the more rapid inactivators of isoniazid are tabulated. In the low-isoniazid–PAS group 55.9 per cent of the cavities closed after five to six months. After a similar period of

TABLE 15

MODERATE TO MARKED ROENTGENOGRAPHIC IMPROVEMENT

(All Cases)

	High Isoniazid–PAS Group		Low Isoniazid–PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent Improved	Per Cent Improved	Number of Cases
3	113	53.5	55.0	91
5–6	109	86.2	81.5	76

TABLE 16

MODERATE TO MARKED ROENTGENOGRAPHIC IMPROVEMENT

(Moderately Advanced Cases)

	High Isoniazid–PAS Group		Low Isoniazid–PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent Improved	Per Cent Improved	Number of Cases
3	90	61.1	54.6	75
5–6	88	87.5	81.0	63

TABLE 17

MODERATE TO MARKED ROENTGENOGRAPHIC IMPROVEMENT

(Far Advanced Cases)

	High Isoniazid–PAS Group		Low Isoniazid–PAS Group	
Number of Months of Treatment	Number of Cases	Per Cent Improved	Per Cent Improved	Number of Cases
3	23	52.2	56.2	16
5–6	21	81.0	84.5	13

time, 42.8 per cent of the cavities closed in the high-isoniazid–PAS group. The slower inactivators of isoniazid are represented in table 14. In this table the situation was reversed. After five to six months, more cavities closed in the high-isoniazid–PAS group (49 per cent) than in the low-isoniazid–PAS group (38.7 per cent).

Moderate to marked roentgenographic improvement is tabulated in tables 15 through 17. There appears to be no marked difference in improvement after three or five to six months when all of the cases, the moderately advanced cases and the far advanced cases, are compared. In all comparisons in both treatment groups, moderate to marked roentgenographic improvement varied between 81–87.5 per cent after five to six months.

Discussion

There are certain factors within the classification of cases in both the high-isoniazid–PAS and low-isoniazid–PAS groups that tend to weight this study in favor of the high-isoniazid–PAS group. In table 1, the division of cases is not dissimilar except for cavitary cases. There are 9.6 per cent more cavitary cases and 20.6 per cent more multiple cavitary cases in the low-isoniazid–PAS group. In table 2, 45.6 per cent of the cases in the low-isoniazid–PAS group had serum concentrations of isoniazid which were below 0.6γ per ml. after six hours. Only 22 per cent of the cases in the high-isoniazid–PAS group had similarly low serum isoniazid concentrations. These aforementioned factors suggest that better treatment results might be expected in that group of patients who were treated with high doses of isoniazid.

In general, the results as tabulated herein indicate that a very slightly higher percentage of cases in the high-isoniazid–PAS group fared better after five to six months of treatment. A closer analysis indicates that this difference between the two treatment groups is not significant.

It is possible that the higher percentage of slow inactivators of isoniazid in the high-dose group and the higher percentage of cavitary cases in the low-isoniazid group could have accounted for the slight percentage in favor of the high-isoniazid group of patients. Excluding comparisons based on serum isoniazid concentrations (tables 13 and 14), the absolute percentage differences between the high- and low-isoniazid groups varied between 1.6 and 7.1 per cent in all phases of evaluation. With specific reference to tables 13 and 14, the percentage of cavities that were closed after five to six months was the reverse of what one would have predicted. Among the more rapid inactivators of isoniazid (table 13), 13.1 per cent more cavities closed in the low-isoniazid–PAS group. Among the slower inactivators of isoniazid (table 14), 10.3 per cent more cavities closed in the high-isoniazid–PAS group. It appears, therefore, that the rate of closure of cavities on any dosage of isoniazid is not related to the concentration of free isoniazid in the serum as determined microbiologically six hours after an oral test dose of the drug.

The lack of evidence of the superiority of the higher dosage of isoniazid observed in the present study is worthy of comment. Several possible explanations exist. The dosage of isoniazid in the high-dosage group was only three to four times greater than in the low-dosage group, and it is possible that pulmonary tuberculosis in man is not a suitable test model for detection of such dosage differences (7–9). So many variables exist, such as differences in the underlying pathology in two lesions presenting a similar roentgenographic appearance, different rates of isoniazid inactivation, possible antagonism by pyridoxine (7) and, presumably, as yet undetermined factors of host resistance. These variables might nullify any over-all detectable difference in treatment results in the group of patients presented herein. Despite the inability to demonstrate the point in actual trial, the writers believe that there is a small group of patients whose disease is better treated on higher doses of isoniazid. These include patients who inactivate isoniazid rapidly, those with cavitary disease, and previously untreated patients who have had productive pulmonary lesions for a long period. Perhaps, if a large group of patients with the above three characteristics were studied, a difference between high and low doses of isoniazid might be detected.

Summary

The clinical results observed in 204 patients have been tabulated with respect to reversal of infectiousness, closure of cavities, and roentgenographic improvement. High doses of isoniazid (10 to 20 mg. per kg. daily), PAS (12 gm. daily), plus pyridoxine (100 mg. daily) were administered

to 113 patients. Ninety-one patients received low or conventional doses of isoniazid (300 mg. daily) plus PAS (12 gm. daily). Patients in both treatment groups had pulmonary lesions that were similar in type except that there were slightly more cavitary cases and, particularly, more multiple cavitary cases, in the low-isoniazid–PAS treatment group.

When isoniazid–PAS is administered to patients with pulmonary tuberculosis, no significant clinical advantage seems to result from high doses of isoniazid. The difference in results between both groups varied between 1.6 and 7.1 per cent, and most frequently was less than 5 per cent. When cavity closure rates were compared with concentrations of free isoniazid in the serum there appeared to be no correlation between the two.

With respect to the case material reported herein, the use of high doses of isoniazid–PAS accompanied by pyridoxine is not superior to conventional 300-mg. daily doses of isoniazid–PAS.

Sumario

Justipreciación de la Quimioterapia en la Tuberculosis Pulmonar. Las Dosis Altas de Isoniacida–PAS Comparadas con las Dosis Bajas de Isoniacida–PAS

Los resultados clínicos observados en 204 enfermos han sido tabulados con respecto al viraje a negativos de los cultivos positivos para el *M. tuberculosis*, el cierre de cavernas y la mejoría en las radiografías torácicas. A 113 enfermos se les administraron dosis altas de isoniacida (10 a 20 mgm. diarios por kg. de peso), PAS (12 gm. diarios) y además piridoxina (a dosis de 100 mgm. diarios). Noventa y un enfermos recibieron dosis bajas o convencionales de isoniacida (300 mgm. diarios) y además PAS (12 gm. diarios). En ambos grupos terapéuticos, los enfermos tenían lesiones pulmonares que eran semejantes en cuanto a forma, salvo por haber unos pocos más casos cavitarios—y en particular cavitarios múltiples—en el groupo terapéutico bajo en isoniazida–PAS.

Cuando se administra isoniacida–PAS a enfermos de tuberculosis pulmonar, no parece obtenerse mayor ventaja clínica con dosis altas de isoniacida. La diferencia en los resultados entre ambos grupos varió entre 1.6 y 7.1 por ciento, siendo más frecuentemente menor de 5 por ciento. Cuando se compararon los coeficientes de clausura de cavernas con las concentraciones de isoniacida libre en el suero, no pareció haber la menor correlación entre las dos cosas.

Con respecto al grupo de casos aquí considerados, el use de dosis altas de isoniacida–PAS acompañado de piridoxina no es superior al de dosis convencionales de 300 mg. diarios de isoniacida–PAS.

Resume

Une évaluation de la chimiothérapie dans la tuberculose pulmonaire. Hautes doses d'isoniazide–PAS comparées à des petites doses d'isoniazide–PAS

Les résultats observés chez 204 malades ont été notés en considérant la conversion vers le négatif des cultures positives pour *M. tuberculosis*, la fermeture des cavernes, et une amélioration des radiographies de poitrine. On administra à 113 malades des hautes doses d'isoniazide (10 à 20 mg par kg par jour), de PAS (12 gm par jour), plus de la pyridoxine (100 mg par jour). Quatre vingt-onze malades reçurent des petites doses ou des doses courantes d'isoniazide (300 mg par jour), plus du PAS (12 gm par jour). Les malades dans les deux groupes de traitement avaient des lésions pulmonaires qui étaient d'un type semblable à part qu'il y avait légèrement plus de cas cavitaires—et en particulier plus de cas avec des cavernes multiples—dans le groupe de traitement avec des doses faibles d'isoniazide–PAS.

Quand on administra de l'isoniazide–PAS aux malades ayant une tuberculose pulmonaire, aucun avantage clinique notable ne sembla résulter des hautes doses d'isoniazide. La différence de résultats entre les deux groupes varia de 1, 6 à 7,1%, et le plus fréquemment, fut de moins de 5%. Quand on compara le nombre de cavernes fermées avec des concentrations d'isoniazide sans sérum, il ne sembla pas qu'il y eut de rapport entre les deux.

En ce qui concerne le "matériel" des cas rapportés içi, l'emploi des hautes doses d'isoniazide–PAS associés à la pyridoxine n'est pas supérieur aux doses journalières courantes de 300 mg d'isoniazide–PAS.

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SPECIFICITY OF MYCOBACTERIAL SENSITINS[1,2,3]

I. Studies in Guinea Pigs with Purified "Tuberculin" Prepared from Mammalian and Avian Tubercle Bacilli, *Mycobacterium Balnei*, and Other Acid-fast Bacilli

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Introduction

Previous studies on the specificity of "tuberculin" (sensitin[4]) prepared from various acid-fast bacilli (1–12) have shown that mycobacterial sensitins possess a certain type or species specificity. This is manifest by the fact that humans or animals infected with a given Mycobacterium usually show stronger reactions to sensitin produced from homologous mycobacteria than to sensitin produced from other species.

These findings, in combination with the practical problems of "nonspecific" tuberculin reactions, presumably cross reactions (3, 4, 10), and the increasing interest in the pathogenic significance of unclassified acid-fast bacteria form the basis for some broader experimental studies carried out in guinea pigs to examine the specificity of purified sensitin prepared from various acid-fast bacilli.

The present paper describes a method for measurement of the specificity of purified sensitin in sensitized guinea pigs, and the findings obtained with 16 sensitin preparations are reported. The sensitins were preparations from 4 human strains of tubercle bacilli, 4 bovine strains (including BCG), 3 avian strains, one *Mycobacterium balnei* strain (13), one *M. smegmatis* strain, and 3 other strains of acid-fast bacilli (nonidentified).

A subsequent paper (14) reports the findings with purified sensitin from several other strains of acid-fast bacilli, including photochromogenic and Battey strains and other unclassified strains.

Materials and Methods

Strains: The strains used for the preparation of purified sensitin are as follows:

Nos. 1 (E 9656), 2 (E 1580), 3 (T 3487), and 4 (T 3419): Virulent strains of *Mycobacterium tuberculosis* var. *hominis*, all isolated at the Tuberculosis Department, Statens Seruminstitut.

No. 5 (BCG): A subculture of a strain used in the BCG Department, Statens Seruminstitut, for preparation of BCG vaccine.

No. 6 (5252): A eugonic bovine strain of tubercle bacilli, isolated from a man in 1937 at Statens Seruminstitut.

No. 7 (Vallée): A eugonic, bovine strain of tubercle bacilli.

No. 8 (V 22250): A eugonic variant of a (dysgonic) bovine strain of tubercle bacilli, isolated from a patient in 1948 at Statens Seruminstitut.

No. 9 (Avian V 64): An avian strain of tubercle bacilli isolated from a pig in 1938.

Nos. 10 (Maren Cecilie) and 11 (E. 38686): Avian strains of tubercle bacilli, isolated from patients in 1944 and 1953 at Statens Seruminstitut.

No. 12 (Mycobacterium balnei XVI): Isolated and described by Linell and Nordén (13).

No. 13 (Mycobacterium smegmatis 8159): Obtained from National Type Culture Collection, London.

Nos. 14 (SSC 263) and 16 (SSC 264): Two unclassified strains of acid-fast bacilli isolated by Engbaek, Tuberculosis Department, Statens Seruminstitut, from sputum collected by a WHO tuberculosis survey team in Africa in 1958. Growth of both strains on Löwenstein-Jensen medium was eugonic, with pale yellow pigmented colonies. No. 14 (SSC 263) grows very rapidly on Löwenstein-Jensen medium, whereas No. 16 (SSC 264) grows fairly slowly on that medium.

No. 15 (SSC 212): An acid-fast bacterium isolated in 1955 by Engbaek, Tuberculosis Department, Statens Seruminstitut, from the

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[1] From the Tuberculosis Department, Statens Seruminstitut, Copenhagen, Denmark.

[2] Presented in part at the VIIth International Congress for Microbiology, Stockholm, Sweden, 1958.

[3] Presented in part at the Symposium on "Schwierigkeiten und Möglichkeiten der Art- und Typenbestimmung von Mycobakterien", Borstel, Germany, June, 1959.

[4] The term "sensitin" is suggested for a nonantigenic substance, prepared from a microorganism (virus, bacterium, or fungus), capable of revealing sensitivity of the delayed type evoked by the organism. Human and avian tuberculin, histoplasmin, and coccidioidin are examples of "sensitin."

sputum of a woman. Growth on Löwenstein-Jensen medium was fairly slow and eugonic, with pale yellow pigmented colonies.

Further characteristics of the strains observed by subculturing the strains on Sauton medium are shown in table 1.

Observation of the growth of the strains on Sauton medium was made as follows: The strains were inoculated on ten 250-ml. flasks, each containing 180 ml. of Sauton medium, as modified slightly by Lind (15). The growth of these cultures was observed, and at various intervals two flasks of each strain were sterilized by heating to 105°C. for one hour (streaming steam). After sterilization the dried weight of bacteria and the pH of the culture filtrate were measured for each flask. The dried bacteria were stored at room temperature in sterile glass tubes for use subsequently in the sensitization of animals. The total crop of bacteria for each strain, shown in table 1, is the maximal value observed on the ten flasks of each strain. The pH values shown in table 1 are the minimal values and the values measured in the last culture of each strain to be sterilized. To a certain extent, these values seem to be characteristic for the various species of acid-fast bacteria (14).

Judging by their growth on Sauton medium, strains 14, 15, and 16 could be expected to represent separate species of acid-fast bacteria which are different from mammalian or avian tubercle bacilli and from *Mycobacterium balnei* or *Mycobacterium smegmatis*.

Preparation of purified sensitin: The method used for preparation of purified sensitin was in most respects the same as that used for preparation of purified tuberculin RT 23 (16). Actually, the four preparations of human tuberculin were samples of lots included in batch RT 23 (*see* table 2).

Sauton medium, modified slightly by Lind (15), was distributed in 1-liter quantities into Fernbach bottles with a maximal capacity of 1.5 liters. After incubation at 38°C., the bacterial cultures were sterilized by heating in streaming steam for one hour. The bacteria were removed from the culture filtrate by filtration through filter paper, coarse Seitz filter plates (KO), and Berkefeld candles. The sterile culture filtrate was subsequently concentrated by ultrafiltration against collodium filters. The sensitin in the concentrated culture filtrates was precipitated by the addition of trichloracetic acid to a final concentration of 2 per cent. The precipitate was centrifuged off and washed four times with 2 per cent trichloracetic acid. Dehydration of the sensitin was effected by stirring the precipitate repeatedly with anhydrous ether.

The changes in this procedure, necessitated by the special properties of some of the strains, consisted of variations in the incubation time of the bacterial cultures according to the growth rate of the strain. Furthermore, the pH was adjusted to about pH 8 before heat sterilization of the acid cultures by adding sodium hydroxide. Finally, in some cases, trichloracetic acid was added to a final concentration of 4 per cent, since 2 per cent did not give full precipitation.

The experimental data and the variations in the preparation procedure for the 16 sensitin preparations are shown in table 2.

Biologic Assay of the Sensitins

The 16 sensitins were all injected intradermally into guinea pigs sensitized with the 16 strains. Differences in the specificity of the sensitins were calculated from the size of the homologous and heterologous (cross) reactions (*see* below).

Sensitization of guinea pigs: One hundred and sixty white guinea pigs, weighing from 400 to 500 gm., were used. The animals were sensitized by two simultaneous injections of 0.25 ml. of a freshly prepared bacterial suspension containing 4 mg. of dried heat-killed bacteria per ml. Dried bacteria from Sauton cultures suspended in light paraffin oil, Bayol F as mentioned above, were used for this purpose.

Ten guinea pigs were used for sensitization with each of the 16 strains. These animals were then used pairwise for intradermal testing in five studies with each preparation (*see* under "Experimental design").

Some of the animals were sensitized in connection with preliminary studies performed prior to the present work. The vaccination period, i.e., the time interval between sensitization and intradermal testing, varied from three weeks to seven months (*see* table 4). However, this variation in length of vaccination period had no detectable influence on registration of the specificity of the preparations.

Sensitin dilutions: Dilutions containing 0.02 mg. of sensitin per ml. (100 sensitin units (SU) per 0.1 ml.) and 0.002 mg. of sensitin per ml. (10 SU per 0.1 ml.) were used in the biologic assay. The diluent was phosphate-buffered saline with 0.005 per cent Tween® 80 and 0.01 per cent chinosol (17). Before use the dilutions had been stored at 4°C. from three days to two months.

Experimental design: The assay consisted of 20 studies performed on ten different occasions over the two-month period June to August, 1957. In each study the specificity of 4 sensitins was compared, the combination of sensitins in groups being made according to the design of Cochran and Cox (18, p. 360, plan 12.2) (*see* also table 3). In this way, each pair of sensitins was compared *within* animals, e.g., 1 and 2, 1 and 3, 1 and 4 (Study 11), 1 and 5 (Study 7), 1 and 6 (Study 9), et cetera.

Eight animals were used in each study, 2 of which had been sensitized with each of the four strains used for the preparation of the sensitins being studied. In one study, all of the animals were tested with the same ten dilutions, which

TABLE 1

CHARACTERISTICS OF STRAINS USED FOR PREPARATION OF PURIFIED SENSITIN

Strain		Origin	Growth on Sauton Medium				
Species and Type	Number		Rate*	Total Crop of Bacteria	pH of Culture Filtrate		Characteristics
					Minimal	Terminal	
				mg.			
M. tuberculosis, human	1. E 9656	SSI† (*see* text)	Medium	1,500	5.0	5.0	Pale yellowish pellicle
M. tuberculosis, human	2. E 1580		Medium	1,200	5.5	5.5	Pale yellowish pellicle
M. tuberculosis, human	3. T 3487		Medium	1,300	5.5	5.5	Pale yellowish pellicle
M. tuberculosis, human	4. T 3419		Medium	1,400	5.3	5.3	Pale yellowish pellicle
M. tuberculosis, bovine	5. BCG		Quick	2,000	5.4	5.4	Pale yellowish pellicle
M. tuberculosis, bovine	6. 5252		Quick	2,100	5.0	5.0	Pale yellowish pellicle
M. tuberculosis, bovine	7. Vallée		Quick				Pale yellowish pellicle
M. tuberculosis, bovine	8. V 22250	SSI (*see* text)	Slow	2,000	5.2	5.2	Pale pellicle
M. tuberculosis, avian	9. V 64		Quick	1,600	5.7	6.0	Thick yellowish pellicle
M. tuberculosis, avian	10. Maren Cecilie		Medium	2,800	5.7	5.9	Thick pale pellicle
M. tuberculosis, avian	11. E 38686		Medium	1,900	5.9	7.2	Thick tan pellicle
M. balnei	12. *M. balnei* XVI	Sweden	Slow				Diffuse growth
M. smegmatis	13. NTCC 8159		Very quick	2,200	8.0	9.2	Buff pellicle
Unclassified	14. SSC 263	Africa (*see* text)	Very quick	4,000	6.3	6.5	Very thick pellicle
Unclassified	15. SSC 212	Denmark (*see* text)	Medium	1,200	4.0	4.0	Tan sparse growth
Unclassified	16. SSC 264	Africa (*see* text)	Medium	800	4.1	4.1	Pale growth

* Growth rate: very quick: cultures fully grown before 2 weeks
quick: cultures fully grown from 2–3 weeks
medium: cultures fully grown from 4–6 weeks
slow: cultures not fully grown after 6 weeks

† SSI—Statens Seruminstitut

TABLE 2

EXPERIMENTAL DATA OF PREPARATIONS OF PURIFIED SENSITIN

Strain		Designation of PPD	Volume of Medium (a)	Growth Period	Ml. of NaOH Added to Each Liter of Culture for pH Adjustment*	pH†	Yield		Changes in Preparation Method
Number	Type						Total Mg. (b)	Relative Mg./L. b/a	
			liters						
1. E 9656	human	RT 23/19	100	5 weeks	—	6.8	8,640	85	
2. E 1580	human	RT 23/91	97	5 weeks	—	8.0	8,500	90	
3. T 3487	human	RT 23/33	98	5 weeks	—	7.4	10,790	110	
4. T 3419	human	RT 23/57	100	5 weeks	—	6.0	4,360	45	
5. BCG	bovine	M 18	18	5 weeks	—	7.3	574	30	
6. 5252	bovine	M 3	11	3 weeks	9 ml. ½ N	6.1	889	80	
7. Vallée	bovine	M 36	11	4 weeks	—	8.4	3,336	300	
8. V 22250	bovine	M 55	11	5 weeks	—	8.0	109	10	
9. V 64	avian	M 13	10	3 weeks	—		786	80	
10. Maren Cecilie	avian	M 79	15	4 weeks	15 ml. ½ N	7.7	1,400	95	Precipitation to 4% trichloracetic acid
11. E 38686	avian	M 83	19	6 weeks	5 ml. ½ N	6.5	661	35	
12. *M. balnei* XVI	*M. balnei*	M 57	3	9 weeks	—		366	120	No Seitz filtration
13. NTCC 8159	*M. smegmatis*	M 40	7	17 days	—	9.4	948	135	
14. SSC 263	Unclassified	M 76	19	9 days	—	8.9	2,547	135	Precipitation to 4% trichloracetic acid
15. SSC 212	Unclassified	M 80	20	4 weeks	40 ml. ½ N	6.9	428	20	
16. SSC 264	Unclassified	M 82	20	4 weeks	30 ml. ½ N	6.5	801	40	

* To obtain a good yield, sodium hydroxide was added to the cultures of the strains which gave a low pH after growth.

† pH was measured electrometrically in some of the culture flasks after heat sterilization (and possible addition of sodium hydroxide) of the cultures. Usually pH was measured in 20% of the culture flasks. The figures shown are the averages.

TABLE 3

OUTLINE OF ASSAY

Study Number	Sensitin Numbers (a)	Square Number*	Sensitization of Guinea Pigs	
			Strains	Number
1	9–10–11–12	II	as a	2 with each strain
2	13–14–15–16	II	as a	2 with each strain
3	3– 7–11–15	I	as a	2 with each strain
4	4– 8–12–16	I	as a	2 with each strain
5	3– 8– 9–14	III	as a	2 with each strain
6	4– 7–10–13	III	as a	2 with each strain
7	1– 5– 9–13	I	as a	2 with each strain
8	2– 6–10–14	I	as a	2 with each strain
9	1– 6–11–16	III	as a	2 with each strain
10	2– 5–12–15	III	as a	2 with each strain
11	1– 2– 3– 4	II	as a	2 with each strain
12	5– 6– 7– 8	II	as a	2 with each strain
13	1– 7–12–14	IV	as a	2 with each strain
14	2– 8–11–13	IV	as a	2 with each strain
15	3– 5–10–16	IV	as a	2 with each strain
16	4– 6– 9–15	IV	as a	2 with each strain
17	1– 8–10–15	V	as a	2 with each strain
18	2– 7– 9–16	V	as a	2 with each strain
19	3– 6–12–13	V	as a	2 with each strain
20	4– 5–11–14	V	as a	2 with each strain

* The roman figures refer to square numbers in plan 12,2, Cochran and Cox, p. 360 (18).

were two concentrations (100 SU and 10 SU) of each of the four sensitins and of RT 23.[5]

The ten injections were distributed at random among ten test sites on the sides and backs of the guinea pigs, in order to compensate for possible systematic errors because of differences in sensitivity between sites. Each injection of 0.1 ml. was given intradermally.

Reading of reactions: The reactions were read after twenty-four hours. The longitudinal and transverse diameters of erythema were recorded in millimeters. Each reaction was read by two readers. The figures presented in table 4 are based on the average of the two readings.

To avoid bias in the reading of tests, the dilutions were labeled in a code unknown to the readers.

RESULTS

The average size of reactions obtained with the 16 sensitin preparations in each group of guinea pigs (2 animals per group) and the standard deviation of these figures are shown in table 4.

In practically all groups, the homologous[6] sensitins evoked the stronger reactions, ranging from 14.0 to 21.9 mm. for 2 γ (100 SU). The average size of the homologous reactions varied somewhat for the 16 sensitins, i.e., from 16.3 mm. (No. 14, SSC 263) to 19.0 mm. (No. 13, *M. smegmatis*). Furthermore, the size of the homologous reactions obtained with each sensitin varied from one group to another. The largest variations were seen for sensitin No. 16 (SSC 264), in which the homologous reactions ranged from 14.0 to 21.4 mm. However, these variations mainly reflected variations in the sensitivity level of the animals. This may be seen from the reactions to the reference preparation RT 23 (unpublished).

In a few cases the heterologous preparations have evoked reactions as large as those of the homologous preparations, e.g., Studies 11 and 12. However, this is mainly the case for sensitins prepared from the same type of mycobacteria as used for sensitization of the animals. In other cases, the reactions to the heterologous sensitins are smaller, ranging from 4.3 to 19.0 mm. for 100 SU.

Specificity of Sensitins

The difference in specificity of two sensitin preparations is characterized by the difference between the homologous and heterologous reactions. The difference is calculated as follows:

SPD = $(A_a + B_b) - (A_b + B_a)$, where A_a is the homologous reaction obtained with sensitin A in animals sensitized with strain a. A_b is the heterologous reaction (cross reaction) obtained with sensitin A in animals sensitized with strain b; B_b and B_a denote the homologous and heterologous reactions with B sensitin.

In cases of small or no differences in specificity of two preparations, the heterologous reactions are nearly as large as the homologous, and therefore SPD ~ 0. When there are large differences in the specificity of two preparations, the homologous reactions are much larger than the heterologous, and therefore SPD > 0, e.g., SPD = 15 mm. Examples of the calculations are shown in table 5.

The results with the present 16 sensitin prepar-

[5] The results obtained with RT 23 are not included in this report.

[6] In the remainder of the paper, the term "homologous sensitin" is used in the restricted sense of sensitin prepared from the same strain as was used for sensitization of the animals. Generally, it is used to designate sensitin prepared from an acid-fast bacilli belonging to the same type as the sensitizing strain.

TABLE 4

MEAN REACTIONS (IN MM.) OBTAINED WITH TWO DOSES OF 16 DIFFERENT SENSITINS IN GUINEA PIGS SENSITIZED WITH THE 16 STRAINS USED FOR PREPARATION OF THE SENSITINS

(Homologous reactions are italicized.)

Study Number	Sensitization of Animals		Sensitin							
	Strain	Vaccination Period	9. V 64		10. M.C.		11. E 38686		12 *M. Balnei*	
			2 γ	0.2 γ	2 γ	0.2 γ	2 γ	0.2 γ	2 γ	0.2 γ
		weeks								
1	9. V 64	21	*18.3*	*14.8*	18.6	15.8	20.1	12.6	12.0	7.6
	10. M.C.	4	16.5	12.3	*17.9*	*12.8*	15.2	12.0	11.1	5.8
	11. E 38686	4	15.6	10.3	16.5	12.1	*14.9*	*11.1*	9.9	4.3
	12. *M. balnei*	20	12.1	7.5	14.4	9.4	14.8	8.5	*16.3*	*14.5*
			13. *M. smegmatis*		14. SSC 263		15. SSC 212		16. SSC 264	
2	13. *M. smegmatis*	21	*21.9*	*18.8*	15.5	10.0	13.0	9.3	11.8	7.6
	14. SSC 263	5	11.9	4.3	*16.5*	*11.0*	6.6	4.1	4.3	3.9
	15. SSC 212	5	9.4	5.1	8.4	4.8	*20.1*	*13.8*	11.9	5.4
	16. SSC 264	5	9.8	4.8	9.3	6.5	9.1	4.6	*18.4*	*13.8*
			3. T. 3487		7. Vallée		11. E 38686		15. SSC 212	
3	3. T 3487	21	*16.5*	*12.8*	18.9	12.3	12.9	10.4	11.9	7.0
	7. Vallée	19	14.9	12.0	*20.8*	*16.3*	13.5	8.4	8.5	5.4
	11. E 38686	4	8.3	6.5	13.4	5.9	*19.4*	*12.0*	11.4	5.8
	15. SSC 212	5	9.5	7.9	12.0	7.9	13.6	8.4	*19.6*	*16.1*
			4. T 3419		8. V 22250		12. *M. balnei*		16. SSC 264	
4	4. T 3419	15	*19.1*	*15.6*	17.8	13.5	13.6	8.0	14.8	5.6
	8. V 22250	4	16.5	11.5	*18.6*	*14.6*	11.4	7.9	8.5	5.1
	12. *M. balnei*	20	12.4	10.4	12.1	5.9	*16.0*	*12.0*	8.0	7.5
	16. SSC 264	5	7.0	8.5	8.0	4.3	8.5	5.0	*21.4*	*16.5*
			3. T 3487		8. V 22250		9. V 64		14. SSC 263	
5	3. T 3487	15–21	*17.3*	*13.4*	17.5	12.3	13.6	8.4	13.1	7.5
	8. V 22250	4	17.3	11.4	*19.6*	*13.4*	12.4	8.1	13.5	8.4
	9. V 64	21	13.9	10.8	13.8	9.8	*16.4*	*14.0*	15.4	13.3
	14. SSC 263	5	7.5	5.3	10.0	3.8	10.1	6.4	*17.0*	*11.5*
			4. T. 3419		7. Vallée		10. M.C.		13. *M. smegmatis*	
6	4. T 3419	15	*17.5*	*12.9*	17.8	12.8	11.9	6.8	11.6	5.9
	7. Vallée	19	13.9	8.3	*16.5*	*11.9*	9.1	6.0	9.1	4.3
	10. M.C.	4	9.8	7.0	13.1	9.5	*16.4*	*12.6*	14.3	11.1
	13. *M. smegmatis*	20	12.9	8.4	14.5	8.5	14.8	10.4	*19.1*	*15.9*
			1. E 9656		5. BCG		9. V 64		13. *M. smegmatis*	
7	1. E 9656	16	*15.1*	*12.5*	18.3	12.4	10.8	7.3	12.0	7.8
	5. BCG	3	13.0	7.4	*15.5*	*10.5*	9.6	4.6	8.1	5.1
	9. V 64	22	13.1	10.9	14.8	11.9	*17.9*	*14.3*	13.8	10.5
	13. *M. smegmatis*	21	12.8	9.1	12.4	8.4	12.1	8.1	*15.3*	*12.0*
			2. E 1580		6. 5252		10. M.C.		14. SSC 263	
8	2. E 1580	16	*15.6*	*11.5*	15.1	11.6	12.6	7.8	12.9	7.6
	6. 5252	20	15.3	10.4	*14.6*	*13.3*	11.8	8.3	10.4	5.8
	10. M.C.	5	8.5	4.3	9.6	6.0	*16.3*	*9.8*	11.6	7.5
	14. SSC 263	6	7.0	3.9	7.5	3.9	9.5	5.5	*14.6*	*11.1*
			1. E 9656		6. 5252		11. E 38686		16. SSC 264	
9	1. E 9656	16	*16.6*	*13.3*	15.5	12.1	12.5	9.5	10.4	5.8
	6. 5252	20	14.6	12.5	*16.1*	*13.0*	11.5	9.9	10.3	6.4
	11. E 38686	5	10.5	8.6	12.0	7.8	*16.4*	*12.6*	9.5	4.0
	16. SSC 264	6	6.8	6.0	6.6	3.6	11.5	5.6	*14.9*	*11.4*
			2. E 1580		5. BCG		12. *M. balnei*		15. SSC 21	
10	2. E 1580	6	*16.5*	*8.8*	15.5	12.5	11.4	6.3	10.4	4.1
	5. BCG	10	17.3	11.3	*17.5*	*15.0*	13.1	6.4	11.3	6.5
	12. *M. balnei*	6	13.5	9.3	15.5	9.6	*15.5*	*12.0*	12.0	7.5
	15. SSC 212	6	12.0	9.8	15.6	10.9	12.9	6.6	*15.4*	*12.4*

TABLE 4—*Continued*

Study Number	Sensitization of Animals		Sensitin							
	Strain	Vaccination Period	1. E 9656		2. E 1580		3. T 3487		4. T 3419	
			2 γ	0.2 γ	2 γ	0.2 γ	2 γ	0.2 γ	2 γ	0.2 γ
		weeks								
11	1. E 9656	28	*15.0*	*10.9*	16.1	11.3	15.4	9.0	13.1	8.8
	2. E 1580	5	15.4	10.5	*16.6*	*10.4*	15.9	9.3	14.5	8.8
	3. T 3487	22	16.5	11.8	17.6	14.3	*16.4*	*12.9*	17.5	14.0
	4. T 3419	6	13.8	12.0	15.6	13.3	15.3	14.4	*14.1*	*13.4*
			5. BCG		6. 5252		7. Vallée		8. V 22250	
12	5. BCG	9	*17.1*	*13.4*	17.0	10.6	17.0	13.0	16.8	11.0
	6. 5252	6	16.1	11.8	*16.5*	*10.8*	16.3	12.9	16.4	13.3
	7. Vallée	6	18.3	11.1	17.1	13.0	*18.6*	*15.4*	17.6	14.3
	8. V 22250	11	17.8	11.6	15.1	12.1	16.8	12.9	*17.6*	*12.3*
			1. E 9656		7. Vallée		12. *M. balnei*		14. SSC 263	
13	1. E 9656	6	*17.4*	*12.3*	16.3	12.4	10.0	6.3	12.6	7.9
	7. Vallée	7	14.8	9.5	*18.5*	*14.9*	9.4	5.0	10.3	7.9
	12. *M. balnei*	28	13.8	9.3	13.6	9.3	*16.5*	*10.9*	12.5	8.4
	14. SSC 263	13	9.0	4.6	12.1	7.9	9.9	7.5	*16.1*	*12.9*
			2. E 1580		8. V 22250		11. E 38686		13. *M. smegmatis*	
14	2. E 1580	6	*17.0*	*10.8*	15.9	9.4	14.1	7.5	10.6	4.0
	8. V 22250	7	17.0	11.8	*17.3*	*14.0*	14.4	10.6	15.0	11.9
	11. E 38686	7	9.8	4.1	7.0	4.5	*16.0*	*12.5*	12.3	6.8
	13. *M. smegmatis*	28	7.6	3.8	7.5	5.5	12.0	5.3	*20.3*	*16.5*
			3. T 3487		5. BCG		10. M.C.		16. SSC 264	
15	3. T 3487	7	*15.9*	*13.1*	19.0	15.4	15.4	11.4	12.3	4.4
	5. BCG	10	16.5	12.1	*19.3*	*14.9*	15.6	12.4	12.0	7.8
	10. M.C.	7	13.8	7.6	16.5	11.6	*19.0*	*15.4*	10.6	4.8
	16. SSC 264	13	7.6	4.4	9.4	5.6	10.3	3.6	*14.0*	*10.6*
			4. T 3419		6. 5252		9. V 64		15. SSC 212	
16	4. T 3419	7	*17.5*	*14.9*	17.8	13.6	15.0	12.0	14.0	7.0
	6. 5252	7	16.6	12.5	*19.6*	*15.6*	13.3	5.5	9.9	7.5
	9. V 64	7	14.9	9.9	16.4	11.1	*20.9*	*14.9*	13.9	7.8
	15. SSC 212	13	7.9	4.1	8.1	3.6	8.8	3.3	*16.8*	*14.9*
			1. E 9656		8. V 22250		10. M.C.		15. SSC 212	
17	1. E 9656	6	*19.0*	*13.9*	19.0	11.5	15.3	8.9	11.8	7.1
	8. V 22250	7	16.5	10.9	*18.8*	*13.6*	13.0	6.8	9.6	6.4
	10. M.C.	7	11.5	7.8	10.6	7.1	*17.4*	*15.9*	11.6	7.5
	15. SSC 212	6	5.0	3.8	5.6	3.5	10.3	4.1	*17.0*	*11.9*
			2. E 1580		7. Vallée		9. V 64		16. SSC 264	
18	2. E 1580	7	*17.1*	*14.8*	18.3	14.0	12.4	7.5	8.4	4.4
	7. Vallée	8	16.0	11.8	*19.4*	*16.1*	14.0	11.8	9.4	5.9
	9. V 64	8	11.8	7.5	15.9	12.4	*18.0*	*13.9*	10.8	5.4
	16. SSC 264	8	7.0	6.8	10.3	7.0	8.6	6.9	*15.6*	*11.1*
			3. T 3487		6. 5252		12. *M. balnei*		13. *M. smegmatis*	
19	3. T 3487	7	*20.0*	*14.8*	18.5	12.5	12.0	6.4	13.9	7.6
	6. 5252	7	16.5	11.0	*19.6*	*14.0*	12.1	5.5	11.1	5.3
	12. *M. balnei*	28	11.6	7.4	13.5	8.9	*16.6*	*13.3*	14.1	8.9
	13. *M. smegmatis*	28	9.9	6.8	10.0	6.5	9.9	6.4	*18.4*	*14.6*
			4. T 3419		5. BCG		10. E 38686		14. SSC 263	
20	4. T 3419	8	*14.3*	*11.9*	15.9	12.3	10.6	5.1	9.1	4.6
	5. BCG	7	14.6	10.0	*16.9*	*12.1*	13.0	6.6	13.0	5.8
	11. E 38686	8	10.3	5.4	14.3	8.0	*19.3*	*14.6*	13.1	9.0
	14. SSC 263	7	9.6	6.5	12.5	8.9	14.9	10.6	*17.1*	*12.6*

Each figure is the mean of two reactions (2 animals).
Standard deviation of means = 1.1 mm. by comparison of reactions on the same animal.
Standard deviation of means = 1.9 mm. by comparison of reactions on different animals.

TABLE 5

CALCULATION OF SPECIFICITY DIFFERENCES

Example, Study 1

Sensitin 9 versus 10 (*avian versus avian*)

$$SPD = \tfrac{1}{2}(14.8 + 18.3 + 12.8 + 17.9) - \tfrac{1}{2}(12.3 + 16.5 + 15.8 + 18.6) = 0.30 \sim 0\ mm.$$

Sensitin 9 versus 11 (*avian versus avian*)

$$SPD = \tfrac{1}{2}(14.8 + 18.3 + 11.1 + 14.9) - \tfrac{1}{2}(10.3 + 15.6 + 12.6 + 20.1) = 0.05 \sim 0\ mm.$$

Sensitin 9 versus 12 (*avian versus M. balnei*)

$$SPD = \tfrac{1}{2}(14.8 + 18.3 + 14.5 + 16.3) - \tfrac{1}{2}(7.5 + 12.1 + 7.6 + 12.0) = 12.35 \sim 12\ mm.$$

Sensitin 10 versus 11 (*avian versus avian*)

$$SPD = \tfrac{1}{2}(12.8 + 17.9 + 11.1 + 14.9) - \tfrac{1}{2}(12.1 + 16.5 + 12.0 + 15.2) = 0.45 \sim 0\ mm.$$

Sensitin 10 versus 12 (*avian versus M. balnei*)

$$SPD = \tfrac{1}{2}(12.8 + 17.9 + 14.5 + 16.3) - \tfrac{1}{2}(9.4 + 14.4 + 5.8 + 11.1) = 10.4 \sim 10\ mm.$$

Sensitin 11 versus 12 (*avian versus M. balnei*)

$$SPD = \tfrac{1}{2}(11.1 + 14.9 + 14.5 + 16.3) - \tfrac{1}{2}(8.5 + 14.8 + 4.3 + 9.9) = 9.65 \sim 10\ mm.$$

TABLE 6

TYPE SPECIFICITY OF PURIFIED SENSITINS*

Type of Sensitin→		Human				Bovine				Avian			Others				
	Strain→	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Human	1. E 9656	—	0	1	3	1	2	5	4	9	11	9	9	7	12	17	14
	2. E 1580	0	—	0	1	1	1	4	3	12	10	10	6	19	11	8	16
	3. T 3487	1	0	—	−2	0	5	4	3	7	8	11	14	15	13	14	13
	4. T 3419	3	1	−2	—	1	4	3	4	8	12	14	9	13	13	16	18
Bovine	5. BCG	1	1	0	1	—	1	3	2	9	6	11	8	10	9	8	12
	6. 5252	2	1	5	4	1	—	1	0	12	9	9	12	17	13	19	14
	7. Vallée	5	4	4	3	3	1	—	1	7	10	14	12	14	12	20	15
	8. V 22250	4	3	3	4	2	0	1	—	10	14	12	12	14	13	18	23
Avian	9. V 64	9	12	7	8	9	12	7	10	—	0	0	12	7	7	17	14
	10. Mar. Cec.	11	10	8	12	6	9	10	14	0	—	0	10	7	9	14	15
	11. E 38686	9	10	11	14	11	9	14	12	0	0	—	10	15	8	14	12
Others	12. *M. balnei*	9	6	14	9	8	12	12	12	12	10	10	—	12	9	8	18
	13. *M. smegmatis*	7	19	15	13	10	17	14	14	7	7	15	12	—	13	19	19
	14. SSC 263	12	11	13	13	9	13	12	13	7	9	8	9	13	—	19	18
	15. SSC 212	17	8	14	16	8	19	20	18	17	14	14	8	19	19	—	18
	16. SSC 264	14	16	13	18	12	14	15	23	14	15	12	18	19	18	18	—

* The table shows the differences (in mm.) between homologous and heterologous reactions obtained in guinea pigs sensitized with the strains used for preparation of the sensitins. The figures are based on the observations shown in table 4. For details of calculation, *see* text.

(To make reading of the table simpler, all figures are shown twice)

Standard deviation of figures = 1.4 mm. for SPD $\leqq$ 3 mm.
Standard deviation of figures = 2.2 mm. for SPD $>$ 3 mm.

ations are shown in table 6.[7] Only very small differences in specificity are seen *within* the groups of human, bovine, and avian tuberculins; for the human tuberculins the range is −2 to 3 mm.; for the bovine, 0 to 3 mm.; and for the avian, 0 mm.

The differences found between human and bovine tuberculins (0 to 5 mm.) are a little larger than the differences within the two groups. BCG tuberculin shows slightly smaller differences than the other bovine tuberculins as compared with the human.

One the other hand, large differences (6 to 14 mm.) are seen between tuberculins from mammalian and avian strains.

Further, balnei sensitin, smegmatis sensitin, and the sensitin from the three unclassified strains show different specificities from those of mammalian and avian tuberculin (SPD ranges from 6 to 23 mm.) and different from each other, SPD ranging from 8 to 19 mm.

Discussion

Several studies have been published previously on the specificity of tuberculin prepared from *human*, *bovine*, and *avian* tubercle bacilli.

In investigations with tuberculin prepared from *different human* strains, no differences in specificity were revealed (9, 19, 20). In some studies, tuberculin prepared from *bovine* strains has had practically the same specificity as tuberculin from human strains (8, 9, 21, 22). In others (23–25), bovine tuberculins gave slightly smaller cross reactions than human tuberculin when tested in animals sensitized with avian tubercle bacilli or other nonmammalian acid-fast bacilli. Pronounced differences in the specificity of *avian* and human tuberculin were reported in a series of works (2, 5–12, 19, 26–28).

Somewhat deviating results were obtained by Baisden, Larsen, and Vardaman (23) and by Svenkerud (29). Baisden and co-workers (23) found it easier to distinguish bovine from human infection in cattle than from avian, phlei, or Johne sensitization by testing with bovine tuberculins, and they were unable to explain these unexpected results. Svenkerud (29) reported the following conclusions from his studies on the specificity of heat-concentrated synthetic medium tuberculins prepared from human, bovine, and avian strains:

> Tuberculin prepared from different human strains may have a different specificity, just as bovine tuberculins prepared from different bovine strains may have different qualitative properties. It may happen that human strains may produce a tuberculin which reacts strongly on avian sensitized animals. Among tuberculins from bovine cultures, some may be found which react very much like the standard type of human tuberculin while others are more related to avian tuberculins.

Due to the great practical importance of possible specificity differences between human tuberculin and *BCG tuberculin*, several investigations have been devoted to this topic. Kurakane and Takasaki (30) and Dedebas (31) found no difference in the specificity of BCG tuberculin and human tuberculin. However, from other studies (8, 32–41), it may be concluded that there are small differences in the specificity of BCG tuberculin and human tuberculin, but that these are too small to be of any clinical interest. One exception, however, is the work of Ohtomo, Takeya, and Toda (42), who made unheated tuberculin preparations from human tubercle bacilli and from BCG, and found large differences in specificity. These observations have not been confirmed by others as yet.

According to Linell and Nordén (13), the specificity of purified sensitin prepared from *Mycobacterium balnei* has previously been tested by Green and Paterson. *M. balnei* sensitin showed a different specificity from that of human tuberculin.

Jensen and Lind (9) found distinct differences in the specificity of sensitin prepared from 5 *saprophytic acid-fast strains* and avian and mammalian tuberculin. The 5 saprophytic sensitins could be divided roughly into two groups, viz., saprophytes isolated from man and saprophytes isolated from waterways, but there were also some differences between the individual saprophytes within each of these two groups.

[7] The standard deviation of SPD was calculated from the differences between two determinations of each specificity difference originating from observations on the first and second animals in each group (each group consists of 2 animals).

A graphic evaluation showed that the standard deviation was smaller for SPD $\leq$ 3 mm. than those for SPD $>$ 3 mm. The standard deviation was, therefore, calculated separately for SPD $\leq$ 3 mm. (SD = 1.4 mm.) and for SPD $>$ 3 mm. (SD = 2.2 mm.).

In the present studies, practically no differences in specificity were found in purified tuberculin from 4 human strains and from BCG. Tuberculins from 3 other bovine strains were more closely related to each other than to the human preparations. No differences were found in 3 avian preparations, but they were all different from the mammalian tuberculins and from *M. balnei* sensitin. Four preparations obtained with other acid-fast strains, including one strain of *M. smegmatis*, were different from each other and were also different from the other preparations tested.

Further studies performed according to the same method (unpublished observations) with six human (25, E5M10; 26, U 1921; 87, Afr. 5121; 88, Afr. 5122; 90, Afr. 5124; 91, Afr. 5126), one bovine (27, T 10620), and one avian tuberculin (28, E 26361) have shown that these tuberculins possess the same specificity as the other tuberculins of the same types. Furthermore, none of the sensitins of the more than 40 prepared from nonmammalian strains has shown the same specificity as the mammalian tuberculins (14).

The conformity within these findings and their agreement with the majority of the previous observations of others show that the present method is suitable for characterization of the type specificity of sensitins.

The main reason for using the specificity differences for characterization of the specificity in the present work instead of the specificity factors as employed by Green (8), McIntosh and Konst (24), and Baisden, Larsen, and Vardaman (23), or the relative potency on heterologous animals (9) is that the calculations are simpler.

In the present study, as in some of the others, the animals have been sensitized by injection of dried, heat-killed bacteria suspended in paraffin oil. The results agree with those obtained by others who used living bacilli for sensitization of the animals. Sensitization with killed bacteria in oil has two important advantages over sensitization with living bacteria. The animals maintain a high level of sensitivity for a long time, no fall in sensitivity having been seen up to one year after vaccination (unpublished observations). Furthermore, sensitization from one sample of bacteria can be performed for a very long period without risk of variations in the biologic and antigenic properties of the strains in the course of series of experiments.

Preparation of purified sensitin from various acid-fast bacteria and study of the specificity of such preparations may be useful in three respects. The studies seem to be of value in classification of acid-fast bacilli (6, 7, 14). In addition, the preparations may be used for skin-testing persons from whom unclassified acid-fast strains of unknown pathogenicity have repeatedly been isolated (43). Finally, the preparations may be of value for detecting and finding the cause(s) of "nonspecific" tuberculin reactions in man (3, 4) and in animals (10). A thorough knowledge of the specificity of the preparations used, especially of the presence of common components in preparations from different strains or the possible identity of such preparations, naturally is of great value in interpreting the results of such comparative studies.

In addition to being applicable to studies on the specificity of mycobacterial sensitins, the present method might be useful for studying the specificity of other sensitins, when cross reactions are known to present problems, e.g., extracts of fungi such as histoplasmin, coccidioidin, blastomycin, et cetera (44–46). Of course, the experimental design must be modified to meet the special conditions, e.g., number of preparations, required accuracy of the experiments, et cetera.

Summary

Purified tuberculin ("sensitin") has been prepared from 16 strains of acid-fast bacilli. The specificity of the preparations has been studied by comparative intradermal tests in guinea pigs sensitized with the 16 strains.

No difference was found in the specificity of tuberculin prepared from BCG and from 4 strains of human virulent tubercle bacilli. The specificity of tuberculin from 3 bovine strains was essentially the same as that of the human tuberculins.

The specificity of 3 avian tuberculins was different from that of the mammalian preparations. No differences were seen between the three avian preparations.

Sensitin from *Mycobacterium balnei* and from 4 other acid-fast bacilli, including one strain of *Mycobacterium smegmatis*, showed specificities which were different from each other and from that of mammalian and avian tuberculins.

Sumario

Especificidad de la Sensitina Micobacteriana. I. Estudios en Cobayos con "Tuberculina" Purificada Preparada de Bacilos Tuberculosos de Mamífero y Aviarios, del Mycobacterium Balnei y de Otros Bacilos Ácidorresistentes

Preparada tuberculina purificada ("sensitina") de dieciséis cepas de bacilos ácidorresistentes. Se estudió la especificidad de las preparaciones obtenidas con intradermorreacciones comparadas en los cobayos sensibilizados con las dieciséis cepas.

No se observó diferencia alguna en la especificidad de la tuberculina preparada del BCG y de cuatro cepas de bacilos tuberculosos humanos virulentos. La especificidad de la tuberculina derivada de tres cepas bovinas fué esencialmente idéntica a la de la tuberculina humana.

La especificidad de tres tuberculinas aviarias fué distinta de la de las preparaciones de mamífero. No se notaron diferencias entre las tres preparaciones aviarias.

La sensitina del *Mycobacterium balnei* y la preparada de otros bacilos ácidorresistentes, incluso una cepa del *Mycobacterium smegmatis*, revelaron especificidades que fueran distintas de las de las tuberculinas de mamífero y aviarias.

Resume

Spécificité des sensitines mycobactériennes: I. Etudes chez des cobayes avec une "tuberculine" purifiée, préparée à partir de bacilles tuberculeux de mammifères et d'oiseaux de Mycobacterium balnei, et d'autres bacilles acido-résistants

On a préparé une tuberculine purifiée ("sensitine") à partir de seize souches de bacilles acido-résistants. On a étudié la spécificité de ces préparations par des examens intradermiques comparés, chez des cobayes sensibilisés aux seize souches.

On n'a trouvé aucune différence entre la spécificité de la tuberculine préparée à partir du BCG et celle des quatre souches de bacilles tuberculeux humains virulents. La spécificité de la tuberculine des trois souches bovines fut essentiellement la même que celle des tuberculines humaines.

La spécificité des trois tuberculines aviaires fut différente de celle des préparations de mammifères. On ne vit aucune différence entre les trois préparations aviaires.

La sensitine de *Mycobacterium balnei* et celle de quatre autres bacilles acido-résistants, y compris une souche de *Mycobacterium smegmatis*, montra des spécificités qui furent différentes de celles des tuberculines de mammifères et d'oiseaux.

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SPECIFICITY OF MYCOBACTERIAL SENSITINS[1,2,3]

II. Studies in Guinea Pigs with Purified Sensitin Prepared from Unclassified Acid-fast Bacilli

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INTRODUCTION

In a previous report (1), a method was described for studying the specificity of purified sensitin[4] prepared from different acid-fast bacteria. In the present paper, this method has been used with sensitins prepared from a series of unclassified strains of acid-fast bacteria.

The organisms used for preparation of purified sensitin include 10 photochromogenic strains isolated in various countries; 2 Battey strains and 2 other unclassified strains isolated in the United States; 12 unclassified strains isolated in Denmark; 8 unclassified strains originating in Africa, and one organism originally classified as *Mycobacterium tuberculosis* but later (2) included in the species *Mycobacterium smegmatis*.

The photochromogenic and Battey strains were isolated in different laboratories (3–7). These organisms are claimed to possess some pathogenicity for humans. The photochromogenic acid-fast organism, *Mycobacterium kansasii* (8) is characterized by its ability to become colored only when exposed to light.

The two Indian strains were among some isolated in 1947–1948. Purified sensitin was prepared from the strains at Statens Seruminstitut in 1949–1950. The results of field studies with Mantoux testing of humans with one of these preparations have been published previously by Edwards and Krohn (9).

The Danish unclassified acid-fast bacteria include a series of unclassified strains isolated at Statens Seruminstitut in the course of routine work. Two of these have been described in detail previously (10, 11), and further details concerning others of these strains will be published separately (12).

The African unclassified acid-fast bacteria are a series of unclassified strains isolated from sputum specimens collected by a WHO tuberculosis survey team working in Africa. The specimens were sent to Statens Seruminstitut for culture. The morphology of some of these strains by culture on Löwenstein-Jensen medium was quite similar to that of human tubercle bacilli.

It was not intended that the strains chosen should be representative of the unclassified strains which occur most frequently. Selection was made on the basis of various aspects, e.g., presumed or known pathogenicity (photochromogenic, Battey, and some Danish strains); previous studies carried out with sensitin produced from identical or similar strains (Indian and Battey strains); geographical origin (African strains, from regions with low-level tuberculin sensitivity); or strains of particular immunologic interest (*Mycobacterium* X, an unclassified acid-fast organism isolated in the United States from mice and originally (13) claimed to be able to induce increased resistance to tuberculosis but no tuberculin sensitivity).

MATERIALS AND METHODS

Strains: The strains used for preparation of purified sensitin are shown in table 1. From the point of view of characterization of the strains, the origin and a few characteristics observed by growing the strains on Sauton medium are given in the table. Observation of growth of the strains on Sauton medium was made as described previously (1).

It may be seen from the table that there are large differences in the growth of the various organisms, although similarities between some of the strains can also be seen.

Preparation of purified sensitin: The prepara-

[1] From the Tuberculosis and Statistical departments, Statens Seruminstitut, Copenhagen, Denmark.

[2] Presented in part at the VIIth International Congress for Microbiology, Stockholm, 1958.

[3] Presented in part at the Symposium on "Schwierigkeiten und Möglichkeiten der Art- und Typenbestimmung von Mycobakterien," Borstel, Germany, June, 1959.

[4] The term "sensitin" is suggested for a nonantigenic substance, prepared from a microorganism (virus, bacterium, or fungus), capable of revealing sensitivity of the delayed type evoked by the organism. Human and avian tuberculin, histoplasmin, and coccidioidin are examples of "sensitin."

TABLE 1

CHARACTERISTICS OF STRAINS USED FOR PREPARATION OF PURIFIED SENSITIN

Strains		Origin	Growth on Sauton Medium					
Group	Number		Rate*	Total Crop of Bacteria	pH of Culture Filtrate		Color†	Other Characteristics
					Minimal	Terminal		
				mg.				
Photochromogenic	30. Goss	Young (7)	Quick	3,800	5.0	5.6	Y	
	34. 0823 P	Selkon *et al.* (6)	Quick	3,900	5.6	6.4	Y	Convoluted pellicle
	35. 0735	Selkon *et al.* (6)	Medium	1,800	4.7	6.2		Diffuse growth + smooth pellicle
	36. 0823 C	Selkon *et al.* (6)	Slow	1,100	5.7	6.0		Granular pellicle
	39. 1438 P	Selkon *et al.* (6)	Medium	3,100	4.9	7.0	Y	Convoluted pellicle, as 34
	41. 1526 P	Selkon *et al.* (6)	Medium	4,300	5.6	5.6	Y	Convoluted pellicle, as 34
	54. ROW	MRC (4), *see* also (6)	Medium	3,800	5.1	5.9	Y	Lumpy growth
	32. Su 1687 a	Sula	Medium	2,000	5.1	6.3		Pellicle with threads
	37. Su 499 a	Ostry *et al.* (5)	Medium	2,000	4.5	6.8		Granular pellicle, as 36
	33. Cuthbert	Middlebrook	Slow	1,500	4.9	6.1		Irregular pellicle, as 36
Battey	72. Burris (7-1376)	Corpe, *see* (3)	Slow	3,200	5.8	6.8	O	
Battey	77. Rainer (5-1923)	Corpe, *see* (3)	Slow	2,000	5.5	6.1	O	Diffuse growth + grayish + orange pellicle
M. tuberculosis (or M. smegmatis)	24. ATCC 607	Frohman	Very quick	2,200	7.1	7.3	Y	
Unclassified USA	43. Mycobact. X	Suter, *see* (13-16)	Slow	1,500	6.3	6.4	Y	Diffuse growth
Unclassified USA	44. Graham	Tarshis	Medium	2,000	5.8	5.8	Y	Diffuse growth + convoluted pellicle
Unclassified India	21. E.	India, *see* text	Quick	3,000	5.1	5.3	S	
Unclassified India	22. M.	India, *see* text	Medium		5.4	5.4	S	
Unclassified Denmark	17. SSC 208, (CPR)	SSI, see (10)	Slow	2,000	6.5	6.5		Diffuse growth
	19. SSC 213	SSI, see text	Quick	900	5.3	5.4		Granular growth
	20. SSC 214	SSI, see text	Quick	1,500	6.1	6.5		Thick pellicle
	23. SSC 209, V 22147	SSI, see (11)	Slow	200	7.3,	7.3	O	Diffuse growth + orange pellicle, as 77
	29. SSC 210	SSI, see text	Quick	2,400	5.7	6.5	D	Buff pellicle; dark culture filtrate
	31. SSC 201	SSI, see text	Quick	1,500	6.6	7.3	Y	
	40. SSC 211	SSI, see text	Medium	300	5.4	5.7		Pale pellicle
	42. SSC 202	SSI, see text	Medium	400	4.9	4.9		Irregular pellicle, as 36
	64. SSC 215	SSI, see text	Medium	3,100	5.2	5.2	S	
	65. SSC 216	SSI, see text	Slow	200	5.5	5.6		Pale diffuse growth + pellicle
	66. SSC 207	SSI, see text	Quick	2,900	5.8	7.8	D	Buff growth; dark culture filtrate, as 29
	79. SSC 217	SSI, see text	Slow	3,000	5.8	7.0	S	
Unclassified Africa	18. SSC 265	Africa, see text	Medium	400	7.5	7.6		Pale parchment-like pellicle
	48. SSC 266	Africa, see text	Medium	800	4.0	4.1		Pale parchment-like pellicle
	51. SSC 269	Africa, see text	Medium	900	4.0	4.0		Irregular pale pellicle, as 18
	52. SSC 270	Africa, see text	Slow	3,100	6.3	7.4	S	
	67. SSC 298	Africa, see text	Medium	2,500	6.2	6.2	Buff	Buff pellicle
	68. SSC 299	Africa, see text	Medium	1,100	4.8	4.8		Pale pellicle
	70. SSC 302	Africa, see text	Medium	1,400	7.3	7.3	Y	Convoluted pellicle
	71. SSC 301	Africa, see text	Medium	1,200	7.8	7.8	Y	Convoluted pellicle

* Growth rate: cultures fully grown within 2 weeks = very quick; cultures fully grown from 2-3 weeks = quick; cultures fully grown from 4-6 weeks = medium; cultures not fully grown within 6 weeks = slow.

† Color of bacterial culture: Y = yellow; O = yellow-orange; S = orange; D = dark.

tion of purified sensitin from each strain was carried out as described previously (1). Experimental data regarding the individual preparations are shown in table 2, in which the strains have been arranged in numerical order.

Biologic Assay of the Sensitins

The specificity of the sensitin preparations was examined by means of intracutaneous tests in guinea pigs sensitized with homologous and

TABLE 2

EXPERIMENTAL DATA CONCERNING PREPARATIONS OF PURIFIED SENSITIN

Strain		Volume of Medium (a)	Growth Period	Ml. of NaOH Added to Each One-Liter Culture for pH Adjustment*	pH†	Yield		Changes in Preparation Method
Number	Type					Total (b)	Relative b/a	
		(liters)				*mg.*	*mg/L*	
17. SSC 208	Danish unclassified	10	6 weeks	—	7.4	308	30	Precipitation to 4% trichloracetic acid
18. SSC 265	African unclassified	20	7 weeks	—	8.7	2,682	135	
19. SSC 213	Danish unclassified	20	18 days	8 ml. ½ N	8.7	4,721	235	Precipitation to 4% trichloracetic acid
20. SSC 214	Danish unclassified	18	18 days	—	8.7	2,841	160	Precipitation to 4% trichloracetic acid
21. E	Indian unclassified	11	5 weeks	—	5.4	1,243	115	
22. M	Indian unclassified	11	5 weeks	—	5.6	1,054	95	
23. SSC 209	Danish unclassified	19	11 weeks	—	7.9	463	25	
24. ATCC 607		10	4 weeks	—	6.3	1,514	150	
29. SSC 210	Danish unclassified	16	4 weeks	—	6.9	459	30	
30. Goss	Photochromogen	18	4 weeks	20 ml. ½ N	7.3	2,619	145	Heat-sterilized twice, first time without NaOH.
31. SSC 201	Danish unclassified	12	4 weeks	—	9.2	2,544	210	
32. Su 1687a	Photochromogen	18	6 weeks	10 ml. ½ N	6.8	2,129	120	
33. Cuthbert	Photochromogen	14	7 weeks	10 ml. ½ N	7.2	1,589	115	
34. 0523 P	Photochromogen	15	4 weeks	10 ml. ½ N	7.3	1,655	110	
35. 0735	Photochromogen	18	5 weeks	30 ml. ½ N	7.2	3,968	220	
36. 0523 C	Photochromogen	12	9 weeks	20 ml. ½ N	6.7	848	70	
37. Su 499 a	Photochromogen	18	5 weeks	10 ml. ½ N	6.7	1,376	75	
39. 1438 P	Photochromogen	17	4 weeks	10 ml. ½ N	8.1	2,158	125	
40. SSC 211	Danish unclassified	18	9 weeks	20 ml. ½ N	8.3	1,102	60	
41. 1526 P	Photochromogen	18	5 weeks	10 ml. ½ N	6.5	2,380	130	
42. SSC 202	Danish unclassified	18	9 weeks	20 ml. ½ N	6.8	192	10	
43. *Mycobacterium* X	U. S. unclassified	18	11 weeks	—	8.0	517	30	
44. Graham	U. S. unclassified	18	7 weeks	—	7.4	1,229	70	
48. SSC 266	African unclassified	17	8 weeks	20 ml. 1 N	7.0	1,940	115	
51. SSC 269	African unclassified	18	9 weeks	20 ml. 1 N	7.1	2,097	115	
52. SSC 270	African unclassified	17	8 weeks	—	8.0	1,720	100	
54. ROW	Photochromogen	18	5 weeks	—	6.4	2,705	150	
64. SSC 215	Danish unclassified	16	4 weeks	10 ml. ½ N	7.4	1,911	120	
65. SSC 216	Danish unclassified	18	11 weeks	10 ml. ½ N	7.1	1,217	70	
66. SSC 207	Danish unclassified	18	4 weeks	—	6.4	896	50	
67. SSC 298	African unclassified	18	6 weeks	—	7.5	6,290	350	Precipitation to 4% trichloracetic acid
68. SSC 299	African unclassified	18	9 weeks	12 ml. 1 N	7.1	4,781	265	
70. SSC 302	African unclassified	18	8 weeks	—	9.1	5,506	305	
71. SSC 301	African unclassified	17	6 weeks	—	8.6	3,336	195	Precipitation to 4% trichloracetic acid
72. Burris	Battey	18	7 weeks	10 ml. ½ N	7.5	1,860	105	
77. Rainer	Battey	18	8 weeks	15 ml. 1 N	8.1	585	35	
79. SSC 217	Danish unclassified	18	7 weeks	10 ml. ½ N	7.5	1,897	105	

* To obtain a good yield, sodium hydroxide was added to the cultures of the strain which gave a low pH after growth.

† pH was measured electrometrically in some of the culture flasks after heat sterilization (and possible addition of sodium hydroxide) of the cultures. Usually the pH was measured in 20% of the culture flasks. The figures shown are the averages.

heterologous acid-fast bacteria in a way similar to that described previously (1). However, in the present study, each preparation was not compared with each of the others. The main principle in this work is that each sensitin is compared with the other sensitins ("type preparations") which have shown different specificity, including some sensitins studied previously (1). This course is not adopted in a few instances in which some examinations not considered of particular interest have been omitted. On the other hand, supplementary examinations have sometimes been carried out when considered of special interest.

The sensitins found by previous examination to have different specificity and which have been used in the present study are produced from strains 3. T 3487 (human), 10. Maren Cecilie (avian), 12. *M. balnei* XVI, 13. *M. smegmatis* NTCC 8159, 14. SSC 263, 15. SSC 212, and 16. SSC 264. Avian tuberculin prepared from strain 9. V 64 was also included in the present study.

Sensitization of guinea pigs: Five hundred and ninety-eight white guinea pigs, weighing 400 to 500 gm., were divided into forty-five groups. Each group (which consisted of 4 to 24 animals) was sensitized with one of the 45 strains used for preparation of sensitin. Two simultaneous subcutaneous injections were given of 0.25 ml. of a suspension containing 4 mg. of dried, heat-killed bacteria per ml. The bacteria were suspended in light paraffin oil, Bayol F. The animals were sensitized three weeks to eight months before being used for intradermal testing (1).

Sensitin dilutions: Dilutions containing 0.02 mg. of sensitin per ml. (100 sensitin units (SU) per 0.1 ml.) and 0.002 mg. of sensitin per ml. (10 SU per 0.1 ml.) were used in the biologic assay. The diluent was phosphate-buffered saline with 0.005 per cent Tween® 80 and 0.01 per cent chinosol. The dilutions had been stored at 4°C. from one day to one and a half years before use.

Experimental design of biologic assay: The assay consisted of 40 studies performed over a period of one and one-half years (from August, 1957, to January, 1959). The number of sensitins compared in each study varied somewhat, but generally five or eight preparations were compared per study. Examples of the experimental design of the single studies are shown in tables 3 and 4. In all cases, each animal was tested with

TABLE 3

EXPERIMENTAL DESIGN FOR STUDY OF SPECIFICITY OF 5 SENSITINS

Sensitins: Nos. 1, 2, 3, 4, and 5

Sensitization of Animals Strain Number	Number of Animals	Sensitins Applied to Animals				
1	2	1	2	3	4	5
2	2	1	2	3	4	5
3	2	1	2	3	4	5
4	2	1	2	3	4	5
5	2	1	2	3	4	5

TABLE 4

EXPERIMENTAL DESIGN FOR STUDY OF SPECIFICITY OF 8 SENSITINS

Type sensitins: Nos. 1, 2, 3, and 4

Test sensitins: Nos. 5, 6, 7, and 8

Sensitization of Animals Strain Number	Number of Animals	Sensitins Applied to Animals				
1	2	1	5	6	7	8
2	2	2	5	6	7	8
3	2	3	5	6	7	8
4	2	4	5	6	7	8
5	2	5	1	2	3	4
6	2	6	1	2	3	4
7	2	7	1	2	3	4
8	2	8	1	2	3	4

10 sensitin dilutions, i.e., two concentrations (100 SU and 10 SU) of each of 5 sensitins. The 5 sensitins are one homologous (prepared from the strain used for sensitization of the animal) and 4 heterologous.

The ten injections were allocated at random to ten test sites on the sides and backs of the guinea pigs. This was done to compensate for systematic errors which might be evoked by differences in sensitivity between sites. Each injection consisted of 0.1 ml. intradermally.

Reading of reactions: The reactions were read after twenty-four hours. The longitudinal and transverse diameters of erythema were recorded in millimeters. Each reaction was read by two readers. The specificity differences (*see* below) were calculated on the basis of the averages of the two readings. To avoid bias in reading the tests, the dilutions were labeled in a code unknown to the readers.

Calculation of specificity differences: The difference in specificity of two sensitin preparations was calculated as the difference between the homologous and the heterologous reactions as follows:

SPD = $(A_a + B_b) - (A_b + B_a)$ where A_a is the homologous reaction (average sensitin reaction) obtained with sensitin A in animals sensitized with strain a. A_b is the heterologous reaction (average sensitin reaction) obtained with sensitin A in animals sensitized with strain b; B_b and B_a denote the homologous and heterologous reactions with B sensitin.

RESULTS

The results of the calculation of the specificity differences (SPD) are shown in table 5 together with the standard deviation of the figures.[5]

[5] The standard deviation of SPD was calculated from the differences between two determinations of each SPD originating from observations on the first and second animals in each group (each group consists of 2 animals). The calculation of the standard deviations was made on a 33 per cent sample of the studies.

The specificity differences varied from −2 to 20 mm. The sensitins were arranged as far as possible in such order in the table that preparations giving small differences with each other were placed together. Various sensitin preparations (with a very few exceptions noted below) were referred to the same specificity group (denoted A–R) if the SPD for the sensitins was smaller or equal to 3 mm.

None of the tuberculins under study has shown the same specificity as *human tuberculin* (Group A), since the SPD values for all the other preparations, when tested against the human preparation No. 3, were ≧ 4 mm.

Two sensitins, Nos. 17, SSC 208 and 44, Graham were classified as belonging to *Group B* avian tuberculin, the SPD ranging from 0 to 3 mm. in this group.

The 2 sensitins prepared from *Battey strains*, Nos. 72 and 77, had slightly different specificity from avian tuberculin (SPD = 4 to 5 mm.), but were the same as each other (SPD = 1 mm.) and, therefore, had been relegated to a separate *Group C*. This group also included sensitin prepared from two Danish unclassified strains, Nos. 23 SSC 209 and 79, SSC 217 (SPD = 0 to 2 mm.), and from strain No. 43, *Mycobacterium* X (SPD = 1 to 3 mm).

Group D comprised 3 sensitins prepared from the *orange-pigmented unclassified strains* Nos. 21, E, and 22, M (both isolated in India) and No. 64, SSC 215 (isolated in Denmark). Some doubt can be raised for justification for including these three preparations in one group, as No. 21 is quite different from No. 22 (SPD = 5 mm.). However, as both these preparations resembled No. 64 (SPD = 2 mm.), it was decided to group them together. Sensitin No. 21 resembled three of the preparations in *Group C*, viz., Nos. 72, 79, and 43 (SPD = 2 to 3 mm.). However, No. 21 was not included in *Group C* because of its difference from Nos. 23 and 77 (SPD = 7 to 9 mm.).

Sensitin prepared from *another orange-pigmented strain* No. 52, SSC 270, originating in Africa, could not be included in *Group D* (SPD = 5 to 9 mm.), but had to be relegated to a special *Group Q*.

Group E contained 3 sensitins prepared from *rapidly growing unclassified acid-fast strains* isolated in Denmark. Preparations Nos. 19 and 31 both showed SPD = 2 mm. when tested against sensitin No. 20.

Group E came fairly close to *Groups B* (SPD = 3–10 mm.) and *C* (SPD = 4–8 mm).

Group F comprised 4 sensitins prepared from *rapidly growing unclassified acid-fast bacilli originating in Africa*. One of these preparations, No. 14, was among those included in the previously reported study (1). The 3 other preparations, Nos. 67, 70, and 71, showed specificity differences ranging from −1 to 2 mm., when tested against this preparation. *Group F* was clearly distinguishable from *Group E*, although it came nearer to this group (SPD = 4–7 mm.) than to any of the others.

All of the 10 preparations of sensitin from *photochromogenic acid-fast bacilli M. kansasii* can be referred to *Group G*. Within this group, SPD ranged from −2 to 3 mm. *Group G* was clearly distinguishable from *Group A* (SPD = 5–13 mm.) and also from the other groups.

Sensitin from *Mycobacterium balnei*, which organism has been reported (17) to be photochromogenic when cultured at room temperature but nonphotochromogenic when cultured at 37°C. (as has been done in the present study), does not fit into *Group G* or any of the other groups and is placed in a separate *Group H*.

Sensitin prepared from strain No. 24, ATCC 607, gave SPD = 3 mm. when tested against *M. smegmatis* sensitin, prepared from strain No. 13, as previously reported (1). The *M. smegmatis* group was designated *Group I*, and was clearly separate from the other groups.

Groups K and L each comprised 2 sensitins prepared from *unclassified acid-fast strains isolated in Denmark*. The specificity differences for the 2 preparations were 2 mm. and 1 mm. within the groups. *Groups K* and *L* were quite separate from the other groups (SPD = 4–16 mm.), and from each other (SPD = 10–11 mm.).

None of the sensitins under study has shown the same specificity as sensitin from strain 15, which was also included in the first study. This preparation was therefore placed in a separate *Group M*.

The 3 sensitins, Nos. 42, 18, and 68, prepared from unclassified acid-fast bacteria isolated in Denmark or Africa, could not be referred to any of these groups either, and were thus placed in separate *Groups N, P*, and *R*.

Finally 2 sensitins, Nos. 48 and 51, prepared from nonidentified strains of acid-fast organisms from Africa, showed a similar specificity to that seen in preparation No. 16 studied previously

TABLE 5

Type Specificity of Purified Sensitins

The table shows the differences (in mm.) between homologous and heterologous reactions obtained in guinea pigs sensitized with the strains used for preparation of the sensitins. To make reading of the table simpler, all figures* are shown twice.

Sensitin Specificity Group →			A	B				C					D			E			F				G										H	I		K	L	M	N	O			P	Q	R
↓	Strain		3	10	9	17	44	23	72	77	79	43	64	21	22	20	31	19	14	67	70	71	30	32	33	34	35	36	37	39	41	54	12	13	24	29	66	40	65	15	42	16	51	48	18	52	68
A	3. T 3487	*M. tuberculosis* var. *hominis*	—	*8*	7	7	10	11	7	11	5	8	4	8	10	12	7	14	*13*	13	10	13	6	5	13	8	9	13	9	6	7	13	*14*	*16*	15	12	15	8	12	*14*	11	*13*	17	10	17	9	10
B	10. M. C.	*M. avium*	*8*	—	*0*	2	0	6	4	5	4	4	10	9	7	4	3	5	*9*	5	10	5	7	6	7	8	7	4	9	6	6	3	*10*	7	12	11	11	8	11	*14*	9	*15*	5	10	8	6	7
	9. V 64	*M. avium*	7	*0*	—	3		4	5		1			6	6				7				7										*18*	7						*17*		*14*					
	17. SSC 208	Unclassified	7	2	3	—		2	4		3			4	9	5		7	11				5										9	7						6		17			9		
	44. Graham	Unclassified	10	0		—		5					13			10			10				12										9	14		11		11		7	10	12			11	7	
C	23. SSC 209	Unclassified	11	6	4	2	5	—	1	0	2	1	7	7	8	7	4		10	7	4	10	10	8	5	8	13	8	13	11	6	8	6	11	8	11	12	10	6	13	8	16	9	8	9	5	7
	72. Burris	Battey type	7	4	5	4		1	—	1	1	2	6	2	4	4	8		6				6										7	11	11	11				10		11			10		
	77. Rainer	Battey type	11	5				0	1	—	1	3	7	9	12								9																								
	79. SSC 217	Unclassified	5	4	1	3		2	1	1	—	1	7	3	7	5			7				8										7	7		10		7	10	11			10	5	
	43. Myc. X	Unclassified	8	4				1	2	3	1	—	8	2	6	5	4		8				6										7	9	11	10				13		14			9		
D	64. SSC 215	Unclassified	4	10			13	7	6	7	7	8	—	2	2	8	7		10		15		9										8	5	9	11		16	11	13		17			9	9	18
	21. E	Unclassified	8	9	6	4		7	2	9	3	2	2	—	5	8	7		13	5		4	7	7	6	11	7	5	7	6	7	5	7	10	7	13	11	9	10	11	6	14	9	12	8	5	
	22. M	Unclassified	10	7	8	9		8	4	12	7	6	2	5	—	11	6		11	7		8	8	9	10	11	10	9	10	10	7	9	8	11	13	14	12	12	6	15	11	17	13	12	11	8	
E	20. SSC 214	Unclassified	12	4		5	10	7	4		5	5	8	8	11	—	2	2	4	5	5	6	12	11	8	16	12	6	11	9	10	5	7	7	11	11	14	7	4	11	6	13	7	9	7	9	9
	31. SSC 201	Unclassified	7	3				4	8			4	7	7	6	2	—		7	5		4	9	10	6	11	13	8	11	10	8	8	3	4	8	9	11	8	4	12	7	12	8	8	7	8	
	19. SSC 213	Unclassified	14	5		7										2	—		7													11	6						13		18			9		
F	14. SSC 263	Unclassified	*13*	*9*	7	11	16	10	6		7	8	10	13	11	4	7	7	—	−1	2	2	10	12	11	8	12	8	12	14	14	13	*9*	*13*	14	12	11	10	9	*19*	9	*18*	11	14	4	6	10
	67. SSC 298	Unclassified	13	5				7						5	7	5	5		−1	—			11										11	11	7	8				18		15			5		
	70. SSC 302	Unclassified	10	10				4					15			5			2		—		11										7	12		11		10		8	5	10			11	7	
	71. SSC 301	Unclassified	13	5				10						4	8	6	4		2			—	13										10	9	8	10				18		11			9		
G	30. Goss	*M. kansasii*	6	7	7	5	12	10	6	9	8	6	9	7	8	12	9		10	11	11	13	—	0	0	2	1	−1	0	0	2	0	6	10	12	9	15	12	12	10	8	10	8	14	9	9	10
	32. Su 1687a	*M. kansasii*	5	6				8						7	9	11	10		12				0	—	1	3	1	0	1	1		0	4	7	13	10				13		12	12		10		
	33. Cuthbert	*M. kansasii*	13	7				5						6	10	8	6		11				0	1	—			2				2	10	17	11	10				9		14	7		5		
	34. 0523 P	*M. kansasii*	8	8				8						11	11	16	11		8				2	3		—	2		3	1			5	9	16	8				14		14			14		
	35. 0735	*M. kansasii*	9	7				13						7	10	12	13		12				1	1		2	—		2	0			8	14	17	9				18		14			11		
	36. 0523 C	*M. kansasii*	13	4				8						5	9	6	8		8				−1	0	2			—				−2	7	13	11	12				8		11	3		5		
	37. Su 499a	*M. kansasii*	9	9				13						7	10	11	11		12				0	1		3	2		—	1			7	12	18	9				16		12			9		
	39. 1438	*M. kansasii*	6	6				11						6	10	9	10		14				0	1		1	0		1	—			9	16	14	7				17		12			9		
	41. 1526 P	*M. kansasii*	7	6				6						7	7	10	8		14				2								—		7	7	9	6				13		12			10		
	54. ROW	*M. kansasii*	13	3				8						5	9	5	8		13				0	0	2			−2				—	9	20	15	12				9		12	5		5		

| | | |

(SPD = −1 to 1 mm.). The sensitin specificity group of these strains was called *Group O*.

Grouping of Acid-fast Bacteria on the Basis of Sensitin Specificity

On the basis of differences in specificity between the various sensitins, as mentioned above and in a previous work (1), it was decided to group the 61 strains referring to the sensitins as shown in table 6.

It may be seen from the table that the BCG strain, originally a bovine strain, comes closer to *Group A*.1 (human) than to *Group A*.2 (bovine). It must be borne in mind, however, that the distinction between specificity *Groups A*.1 and *A*.2 is not very definite.

It may also be seen from the table that all of the *10 strains of M. kansasii* included in the study belong in the one sensitin specificity group (*G*). The *two strains of Battey type* are included in a separate group (*C*) distinguishable from *Group B*. Of the *3 strains originating in the United States*, one (No. 44, Graham) has been allocated to *Group B* (avian), No. 43, *Mycobacterium* X to *Group C* (Battey), and No. 24, ATCC 607 to *Group I* (*M. smegmatis*).

The *two Indian strains* are included, together with another orange-pigmented strain (No. 64) isolated in Denmark, in *Group D*. One of the other *Danish strains* (No. 17, SSC 208) has been placed in *Group B* (avian); and 2 (No. 23, SSC 209 and No. 79, SSC 217) in *Group C* (Battey); while the remaining Danish strains are distributed over five other groups, viz., 3 strains to *Group E*, 2 strains to *Groups K* and *L*, and one strain to *Groups M* and *N*.

The *strains isolated in Africa* did not belong to any of these groups, but 4 belonged in *Group F*, 3 in *Group O*, and one in each of *Groups P*, *Q*, and *R*.

Thus, with regard to some of the groups (*E*, *F*, *K*, *L*, and *O*), there seemed to be a certain

TABLE 6

GROUPING OF ACID-FAST BACILLI* ACCORDING TO SENSITIN SPECIFICITY

Strains	Sensitin Specificity Group																	
	A1. Human	A2. Bovine	B. Avian	C. Battey	D. Orange-pigmented I	E. Unclassified (Denmark)	F. Unclassified (Africa)	G. Photochromogenic	H. *M. balnei*	I. *M. smegmatis*	K. Unclassified (Denmark)	L. Unclassified (Denmark)	M. Unclassified (Denmark)	N. Unclassified (Denmark)	O. Unclassified (Africa)	P. Unclassified (Africa)	Q. Orange-pigmented II (Africa)	R. Unclassified (Africa)
M. tuberculosis var *hominis*	10																	
M. tuberculosis var. *bovis*		4																
BCG	1																	
Mycobacterium avium			4															
M. balnei									1									
M. smegmatis										1								
M. kansasii								10										
"Battey-type"				2														
Unclassified (USA)			1	1														
ATCC 607 (USA)										1								
Unclassified India					2													
Unclassified Denmark			1	2	1	3					2	2	1	1				
Unclassified Africa							4								3	1	1	1

* The table includes also the 24 organisms studied in the previous report (1).

relationship between the specificity group and the geographic origin.

In order to ascertain whether the strains distributed on the basis of differences in sensitin specificity showed other particular characteristics within these groups, the details of the characteristics observed by culture of some of the strains on Löwenstein-Jensen medium are given in table 7. The same table also shows, although in simplified form, the characteristics of the strains observed by culture on Sauton medium, as already shown in table 1.

The strains in *Group C* (Battey type) grew slowly and diffusely on Sauton medium.[6] The organisms had a yellow or orange pigmentation on this medium and were nonpigmented (cream) on Löwenstein-Jensen medium. The organisms in *Groups D and Q* had a strong orange color on both media.

The strains in *Groups E and F* grew rapidly, with characteristic differences between the groups with regard to morphology of colonies on Löwenstein-Jensen medium.

In contrast to the other groups, all of which were fairly homogeneous, the Sauton cultures of the 10 strains of *M. kansasii* (*Group G*) showed fairly wide variations between the strains with regard to growth rate, acidification of culture medium during growth, appearance of bacterial culture, and total bacterial crop. One characteristic common for strains, however, was that they became colored when exposed to light on solid medium. Other common characteristics have been observed by others (*see* Discussion).

The two strains in *Group K* gave very characteristic dark-pigmented cultures on Sauton medium. The colony morphology on Löwenstein-Jensen medium of strain No. 29 was similar to that seen in *Group F*.

The two strains in *Group L* had a characteristic morphology on Löwenstein-Jensen medium resembling that of the dysgonic human strains, although the central elevation was smoother.

The colony morphology of the cultures of the strains in *Groups O* and *R* was very similar to that seen in *Group E*, but the growth rate was slower and the cultures were more pigmented. The strains related to these groups produced a very low pH during culture on Sauton medium.

It was thus evident that in some cases agreement was found when grouping the strains on the basis of sensitin specificity and on the basis of characteristics observed by culture on solid or liquid medium.

[6] By repeated subcultures of the strains on Sauton medium, variants, which grew fairly quickly as surface pellicles, were isolated from three of the strains (Nos. 23, 43, and 77). Purified sensitin was prepared from these variants of strains Nos. 23 and 77. These sensitins had the same specificity as the two purified sensitin preparations Nos. 23 and 77 made from the "original" cultures, as described above.

Discussion

The present study can be evaluated from two angles: (*a*) from the point of view of sensitin specificity with the practical use of the preparations in mind, and (*b*) from the point of view of identification of acid-fast bacteria on the basis of sensitin specificity.

A number of studies have been published previously concerning the *specificity of sensitin* produced with strains which are identical with or nearly related to the strains used here. Examinations have been carried out on both animals (generally guinea pigs) and humans. Some of these works, particularly the specificity studies on human and avian tuberculin, have been referred to previously (1) and will not be mentioned here. The main emphasis will be laid on the experiments on animals, since some of the studies on humans will be discussed further in connection with the publication of the results in humans with the preparations concerned.

Specificity studies with *Battey sensitin* have been carried out by Edwards and Palmer (18), Affronti (19), Edwards, Edwards, and Palmer (20) and Engbaek, Magnusson, and Nielsen (11). Edwards and Palmer (18) found specificity differences between purified sensitin prepared from Battey strains and human purified tuberculin PPD-S by comparative testing with the preparations in tuberculous patients and in patients infected with Battey strains. The homologous preparations gave the stronger reactions in both groups. Affronti (19) reports the preparation method for these sensitins and has observed specificity differences between the preparations in guinea pigs and rabbits. Edwards, Edwards, and Palmer (20) have also found such differences in specificity by tests on guinea pigs sensitized with BCG and Battey strains. Engbaek, Magnusson, and Nielsen (11) have observed differences between the specificity of

TABLE 7

CHARACTERISTICS OF ACID-FAST BACILLI BY CULTURE ON LÖWENSTEIN-JENSEN AND SAUTON MEDIA, ACCORDING TO SENSITIN SPECIFICITY GROUP OF THE STRAINS

Sensitin Specificity Group	Strains			Löwenstein-Jensen Culture (37°C.)*			Sauton Culture (37°C.)			
	Characterization	Number of Strains	Laboratory Numbers	Growth Rate†	Pigmentation	Colony Morphology	Growth Rate‡	Pigmentation	Acidification of Culture Filtrate§	Special Characteristics
A	*M. tuberculosis* var. *hominis*, BCG, and eugonic variants of bovine type	15	1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, 87, 88, 90, 91				m	Pale	++	
B	*M. avium* Unclassified	4 2	9, 10, 11, 28 17, 44	m	Cream	Dysgonic smooth (No. 17)	m/s	Pale or yellow	+/++	
C	Battey type Unclassified	2 3	72, 77 23, 43, 79	m	Cream	Dysgonic to moderately eugonic smooth (Nos. 23 + 79)	s	Yellow or orange	−	Diffuse growth
D	Unclassified (orange-pigmented)	3	21, 22, 64	m	Orange	Moderately eugonic smooth (No. 64)	q/m	Orange	++	
E	Unclassified (Denmark)	3	19, 20, 31	q	Pale	Eugonic, dome-shaped, smooth	q	Yellow	+/++	
F	Unclassified (Africa)	4	14, 67, 70, 71	q	As group A (human)	Eugonic; skittleshaped or flattened, rough to varying degrees	q/m	Pale or buff	−/+	
G	*M. kansasii*	10	30, 32, 33, 34, 35, 36, 37, 39, 41, 54				q/m/s	Pale or yellow	+/++	
H	*M. balnei*	1	12				s			Diffuse growth
I	*M. smegmatis* ATCC 607	1 1	13 24	q			vq	Yellow or buff	−	
K	Unclassified	2	29 66	q	As group A (human); pale yellowish	As group F (No. 29); eugonic smooth (No. 66)	q	Dark	++	

L	Unclassified	2	40, 65	m	Yellowish	As dysgonic human, but with smooth central knob	m/s	Pale	++	
M	Unclassified	1	15	m	Pale yellowish	Eugonic, slightly rough	m	Tan	+++	
N	Unclassified	1	42	m	Cream	Dysgonic to moderately eugonic, slightly rough	m	Pale	+++	
O	Unclassified	3	16, 48, 51	m	Pale yellowish	As group E	m	Pale	+++	
P	Unclassified	1	18	m	Pale	Eugonic, raspberry-shaped, slightly rough	m	Pale	−	
Q	Unclassified (orange-pigmented)	1	52	m	Orange	Dysgonic, smooth	s	Orange	+	
R	Unclassified	1	68	m	Yellowish	As group E	m	Pale	+++	

* When only some of the strains in a group were observed, the numbers of these strains are shown in parentheses.
† q: quick; colonies visible after 2 days.
m: moderate; colonies visible after 7 days.
‡ vq: very quick; cultures fully grown after 2 weeks.
q: quick; cultures fully grown from 2–3 weeks.
m: moderate; cultures fully grown from 4–6 weeks.
s: slow; cultures not fully grown after 6 weeks.
§ −: pH minimum value: 7.0–7.9.
+: pH minimum value: 6.0–6.9.
++: pH minimum value: 5.0–5.9.
+++: pH minimum value: 4.0–4.9.

the present sensitin No. 23 (prepared from a strain which seems to be of Battey type) and human tuberculin, found by skin-testing a group of tuberculous patients and the patient from whom the strain was isolated. Aronson and Kraus (21) observed cross reactions in tuberculous patients to sensitin prepared from various strains, including a Battey strain.

Suter (13) reported that *Mycobacterium* X, i.e., the strain designated No. 43 in this study and here found to be of Battey type, was unable to render guinea pigs sensitive to human tuberculin but, nevertheless, might induce increased resistance to tuberculous infection in such animals. In a later report by Suter and Strain (15), however, reference is made to studies by Boyden according to which guinea pigs infected with *Mycobacterium* X become positive to human tuberculin. Results of immunization experiments performed with the organism and the findings of sensitization against human tuberculin are taken by the writers as evidence of the presence of common antigens in mammalian strains of mycobacteria and in *Mycobacterium* X.

As mentioned previously, one of the present sensitins, No. 22, M, prepared from an *orange-pigmented acid-fast bacterium*, was among those used in comparative studies on "tuberculin" testing in humans by Edwards and Krohn (9). They found evidence of differences in the specificity of the two sensitins, M sensitin being relatively stronger than human tuberculin in persons with low-level tuberculin sensitivity.

In studies with sensitin prepared from *photochromogenic acid-fast bacteria* ("yellow bacillus," *Mycobacterium kansasii*) by Affronti (19), Cohn, Östreicher, and Middlebrook (22), Edwards and Palmer (18), and Nassau and Hamilton (23), evidence is presented of specificity differences between "yellow" sensitin and human tuberculin. Tarshis and Frisch (24) have made similar observations, but Middlebrook and associates (25) suggest that the strains studied by Tarshis and Frisch are not pathogenic mycobacteria of the "yellow bacillus" type encountered by others (25–27). In a study by Pollak and Buhler (28), no evidence of specificity differences between yellow sensitin and human tuberculin was obtained. The dose of yellow sensitin is not mentioned and it may be that it was too small.

According to Runyon (29), a photochromogenic sensitin "luciflavin," prepared and distributed by Cohn and Middlebrook, has been shown to have a marked degree of specificity for photochromogenic infections. A negative finding, however, is reported by Young (7) on the patient from whom the strain Goss (present No. 30) was isolated.

It would seem from previous studies, therefore, that there are differences in the specificity of human tuberculin and "yellow" sensitin but, as mentioned by Edwards and Palmer (18), these may be too small to be recognized easily by comparative testing in humans, and thus are without any direct clinical interest.

The present findings regarding the specificity of sensitin prepared from various acid-fast bacteria thus agree with what has been found previously, although the present findings may be more clear-cut than might have been expected from previous observations. It might also be anticipated that at least some of the preparations would be of value for skin-testing persons suspected of being infected with acid-fast bacteria other than tubercle bacilli.

The *classification of acid-fast bacteria* which can be made on the basis of the specificity of sensitin produced from the various strains (*see* above) is in agreement in many cases with the grouping made by other means, e.g., colony morphology, pigmentation, virulence examination, phage typing, biochemical methods, et cetera. However, it is hardly possible by the present method to distinguish with certainty the human from the eugonic bovine strains. On the other hand, a clear distinction can be made between mammalian and avian strains.

On the basis of sensitin specificity, all of the 10 photochromogenic strains isolated in different laboratories fall into one group. By other methods also (6, 27, 29–33), the photochromogenic strains form a fairly homogeneous group. The name *M. kansasii* (8) is used for this species.

In the majority of instances, the present method seems to be suitable for distinguishing between avian and "Battey" strains, which is not the case with phage typing (34), neutral red reaction (35), enzyme examinations (30), or lipid analysis (32).

Strain No. 17, SSC 208 (CPR), described in detail previously as not being classifiable according to the methods used (11), has subsequently been allotted by Runyon (29) to *"Group III"*

(Battey type), although no information is available as to how this is arrived at. According to the present method, SSC 208 would seem to be an avian strain, while, on the basis of the same method, another Danish unclassified strain, No. 23, SSC 209, V 22147, must be classified as a Battey strain (12). *Mycobacterium* X, which could not be classified according to the methods then used (15), must now also be regarded as belonging to the "Battey" strains. By the present method, strain ATCC 607 (No. 24) seems to belong to the species *Mycobacterium smegmatis*. This is in agreement with observations made by other methods (2, 30, 36). It is therefore considered justified to refer to the strain as *M. smegmatis* 607, as Mayer and co-workers (37) have done.

The orange-pigmented strains examined in this study do not fall into one homogeneous group, but this does not happen with other methods either (17, 30, 38, 39).

On the other hand, in the present study (table 6), a number of other unclassified acid-fast bacteria seem to fall into different characteristic groups, e.g., 4 strains in one group (*F*) and 3 in another group (O). Whether or not it is justifiable to describe some of these groups as separate species is a question which must depend on more comprehensive examination of the strains by current methods also. It should be stressed here that no examination has been made of the relationship between the strains concerned and the species already known, e.g., *M. fortuitum* (40–43), *M. scrofulaceum* (44, 45), *M. paraffinicum* (46), or to known species of the genus Nocardia. However, such studies are now being carried out.

The distribution of acid-fast bacteria into groups A-R should naturally not be regarded as an attempt to group acid-fast bacteria in general, but it is intended merely as a basis for distribution of the strains examined here.

Even though it would thus appear to be possible to group acid-fast bacteria on the basis of specificity studies with sensitin produced from the corresponding strains, it must be emphasized that the method *in its present form* is not suitable for routine determination of acid-fast bacteria, since it involves a great deal of time and effort to produce sensitin from each strain. However, in special cases, it may be useful for classification of acid-fast bacteria difficult to identify by ordinary methods. It is possible that the procedure can be modified to make it usable in routine work, and experiments are in progress to ascertain whether this is the case.

Summary

Purified tuberculin ("sensitin") has been prepared from 37 unclassified strains of acid-fast bacteria, and their specificity (cross reactivity) studied by means of comparative intradermal testing in guinea pigs sensitized with the strains in question.

On the basis of their specificity, the sensitins from 10 photochromogenic strains (*Mycobacterium kansasii*) form one group. The specificity of two Battey sensitins was different from that of mammalian and avian tuberculin and from *M. kansasii* sensitin. Sensitin from strain ATCC 607 had a similar specificity to that of *M. smegmatis* sensitin, and sensitin prepared from "*Mycobacterium* X" had the same specificity as Battey sensitin.

Two sensitins prepared from Indian orange-pigmented strains could be grouped together with sensitin from a Danish orange-pigmented strain, although sensitin from an orange-pigmented strain isolated in Africa did not fit into this group. One of the sensitins prepared from other Danish strains had a similar specificity to that of avian tuberculin; two had the same specificity as Battey sensitin, while the remaining eight preparations could be divided into four groups. None of the 8 sensitins prepared from African strains fitted into any of these groups but formed four separate groups.

On the whole, grouping of acid-fast bacteria on the basis of sensitin specificity correlates well with grouping based on other bacteriologic criteria. The method may be useful for identification of acid-fast bacteria difficult to classify by other means.

Sumario

Especificidad de la Sensitina Micobacteriana. II. Estudios en Cobayos con Sensitina Purificada Preparada de Bacilos Acidorresistentes Inclasificados

Se ha preparado tuberculina purificada ("sensitina") de 37 cepas inclasificadas de bacterias ácidorresistentes y se ha estudiado su especificidad (reactividad cruzada) por medio de intradermorreacciones comparadas en cobayos sensibilizados con las cepas en cuestión.

A base de su especificidad, las sensitinas derivadas de 10 cepas fotocromógenas (*Mycobacterium kansasii*) forman un grupo. La especificidad de las sensitinas Battey fué distinta de la mostrada por la tuberculina mamífera y aviaria y por la sensitina *kansasii*. La sensitina procedente de la cepa ATCC 607 poseía una especificidad semejante a la de la sensitina *smegmatis*, y la preparada del "*Mycobacterium* X" mostró la misma especificidad que la sensitina Battey.

Dos sensitinas preparadas de cepas de pigmentación anaranjada de la India pudieron agruparse con la sensitina derivada de una cepa de pigmentación anaranjada danesa, aunque no pudo acomodarse en este grupo la sensitina preparada de una cepa de pigmentación anaranjada aislada en Africa. Una de las sensitinas preparadas de otras cepas danesas poseía una especificidad semejante a la sensitina aviaria; dos poseían la misma especificidad que la sensitina Battey, en tanto que podía dividirse en cuatro grupos a las ocho preparaciones restantes. Ninguna de las ocho sensitinas preparadas de cepas africanas encajaba en ninguno de estos grupos, sino que formaban cuatro grupos distintos.

En conjunto, la clasificación de las bacterias ácidorresistentes a base de la especificidad de sus sensitinas se correlaciona bien con la clasificación basada en otras pautas bacteriológicas. El método puede resultar útil para la identificación de bacterias ácidorresistentes que son difíciles de clasificar por otros medios.

Resume

Spécificité de la sensitine mycobactérienne. II. Etudes chez des cobayes avec une sensitine purifiée préparée à partir de bacilles acido-résistants non classés

On a préparé une sensitine purifiée, préparée à partir de 37 souches non classées de bactéries acido-résistantes, et leur spécificité (cross-réaction) a été étudiée par des examens intradermiques comparés chez des cobayes sensibilisés avec les souches en question.

En se basant sur leur spécificité, les sensitines de 10 souches photochromogènes (*Mycobacterium kansasii*) forment un seul groupe. La spécificité de deux sensitines Battey fut différente de celle des tuberculines d'oiseaux et de mammifères, et de la sensitine de *M. kansasii*. La sensitine de la souche ATCC 607 eut une spécificité semblable à celle de la sensitine du *M. smegmatis*, et la sensitine préparée à partir de "*Mycobacterium* X" eut la même spécificité que la sensitine Battey.

Deux sensitines préparées à partir de souches indiennes ayant un pigment orange purent être groupées ensemble avec la sensitine d'une souche danoise ayant un pigment orange, bien que la sensitive d'une souche ayant un pigment orange isolée en Afrique n'alla pas avec ce groupe. Une des sensitines préparées à partir d'autres souches danoises eut une spécificité semblable à celle de la sensitine aviaire; deux d'entre'elles eurent la même specificité que la sensitine Battey, tandis que les huit préparations restantes purent être divisées en quatre groupes. Aucune des huit sensitines préparées à partir des souches africaines n'alla dans aucun de ces groupes mais forma quatre groupes séparés.

En gros, le groupement des bactéries acido-résistantes, en se basant sur la spécificité de la sensitine, correspond bien avec le groupement basé sur d'autres critères bactériologiques. La méthode peut être utile pour identifier des bactéries acido-résistantes difficiles à classifier par d'autres moyens.

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EFFECTS OF KILLED TUBERCLE BACILLI (BCG) AND CONSTITUENTS UPON THE RETICULOENDOTHELIAL SYSTEM, THE INTERNAL ORGANS, AND THE DISTRIBUTION OF CIRCULATING LEUKOCYTES OF NORMAL ALBINO MICE[1, 2]

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INTRODUCTION

It has long been recognized and repeatedly confirmed that administration of killed bacilli raises the resistance of the animal host to a subsequent infection with unrelated organisms (1–3). Thus, *Mycobacterium tuberculosis* and certain extracts prepared from it induce in the albino mouse not only antituberculous immunity (4), but also—and long before specific immunity appears—a high level of resistance against such other infective agents as coagulase-positive staphylococci and *Mycobacterium fortuitum* (3). The most potent compounds so far have been obtained by prolonged extraction with methanol from phenol-killed BCG. Their activity, however, can be altered by certain chemical manipulations, notably, short extraction with acid (3), which holds true also for gram-negative bacterial endotoxins (5). Since very little is known about the mode of action of these materials at the present time, it seemed worth while to examine their effects upon normal, noninfected animals.

It has already been demonstrated that, during the early phase of a mycobacterial infection, the ability of the phagocytic cells from the liver and spleen (reticuloendothelial cells) to take up foreign particles injected into the blood stream (granulopectic activity) was greatly modified (6). In a previous communication (7), evidence was produced that killed, acetone-extracted BCG protects albino mice against an intravenous challenge with *Mycobacterium fortuitum*. Simultaneously, a significant rise in granulopectic activity of the reticuloendothelial system was recorded which was still demonstrable two weeks after the initiation of the experiment. It was not possible, however, to evaluate the effect of each stimulus separately, since the mere infection with *M. fortuitum* is able to greatly increase the granulopectic potentiality of the host (8). In the present investigation, not only killed BCG, but a variety of subfractions of proved protective activity (3), were examined for their capacity to stimulate the reticuloendothelial system of normal mice. As it has been observed that conspicuous changes occur in the distribution of circulating leukocytes after administration of fractions extracted from *M. tuberculosis* in rabbits and guinea pigs (9), as well as after intraperitoneal injection of whole, killed BCG in mice (10), and since in both cases typical alterations in the tissues were noticed, a pertinent study is included.

MATERIAL AND METHODS

The following preparations were tested:

(*1*) BCG, strain Phipps (BCG-P), grown for four weeks at 37°C. in Tween®-albumin medium, washed three times with cold acetone, and dried.

(*2*) BCG, residue after acetone extraction: BCG, strain Phipps, extracted with acetone at 37°C. for forty-eight hours.

(*3*) Methanol extract: This material was obtained by extracting the residue of step (*2*) (BCG residue after acetone extraction) with methanol at 56°C. for twenty-four hours. The methanol-soluble fraction was collected by evaporation of the solvent and freeze dried.

(*4*) "pH 10": The residue of step (*3*) was extracted for thirty minutes with 1 N sodium hydroxide at pH 10 and 80° C. The residue was used as preparation "pH 10."

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[2] This work was presented in part at the Third International Symposium of the Society for Research on the Reticuloendothelial System, Rapallo, Italy, August, 1958.

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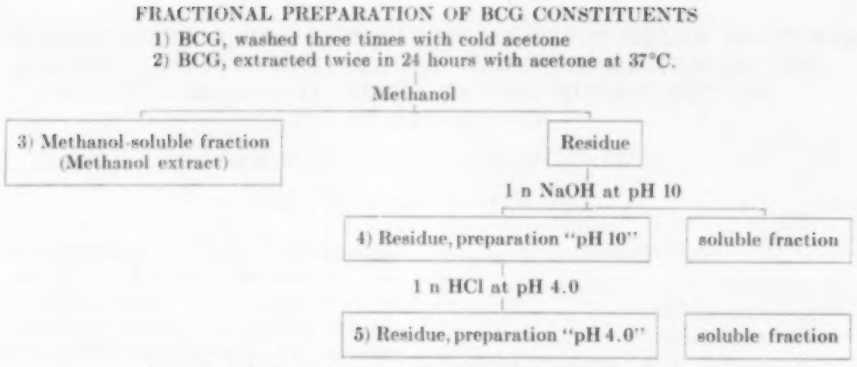

FRACTIONAL PREPARATION OF BCG CONSTITUENTS
1) BCG, washed three times with cold acetone
2) BCG, extracted twice in 24 hours with acetone at 37°C.
Methanol
3) Methanol-soluble fraction (Methanol extract)
Residue
1 n NaOH at pH 10
4) Residue, preparation "pH 10"
soluble fraction
1 n HCl at pH 4.0
5) Residue, preparation "pH 4.0"
soluble fraction

TEXT-FIG. 1. Fractionation of mycobacterial constituents.

(*5*) "pH 4": This substance was obtained by extracting fraction "pH 10" for ten minutes with 1 N hydrochloric acid at pH 4 and 80°C. The residue of this procedure was designated preparation "pH 4."

A schematic diagram of the methods used to isolate the compounds is presented in text figure 1.[6]

The bacillary materials were invariably administered intraperitoneally. For injection, 200 γ of each preparation were suspended in 0.3 ml. of physiologic saline.

As experimental animals, male albino mice of the Swiss Rockefeller strain were used exclusively. They were taken into the experiment at four weeks of age, shortly after weaning, and weighed between 19–21 gm. All were kept in a separate animal room in steel cages in which the sawdust was changed three times weekly, and they received pellets and water *ad libitum*.

Two hundred and eighty mice were divided into six groups at random:

(*1*) The first group of 55 animals served as controls, and received 0.3 ml. of physiologic saline intraperitoneally.

(*2*) The second group of 55 animals received 200 γ of acetone-washed BCG-P.

(*3*) The third group of 40 animals received 200 γ of the preparation "BCG residue after acetone extraction."

(*4*) The fourth group of 40 animals received 200 γ of methanol extract.

(*5*) The fifth group of 45 animals was injected with 200 γ of preparation "pH 10."

(*6*) The sixth and last group of 45 animals was given 200 γ of preparation "pH 4.0."

The activity of the reticuloendothelial system was tested by a method based upon the rate of clearance of carbon particles following intravenous injection of india ink as described in previous papers (7, 8, 11–14). Under the conditions of these experiments and with the proper amount of india ink, the particles are taken up almost exclusively in the liver and spleen. As the results of the technique are influenced by the size of the particles, it is important to use a stable suspension of homogenous size (250 Angström). The standardized preparation used[7] was devoid of toxicity.

At various times after administration of the above listed materials, 7 animals of each group were taken at random; 2 mice were sacrificed exclusively for histologic examination, and 5 were tested in the following manner:

Each mouse received intravenously a dose of 16 mg. of carbon per 100 gm. of body weight. After two, four, six, eight, ten, and fifteen minutes, 0.025 ml. of blood was withdrawn from the retro-orbital venous plexus in the nasal angle of the eye. In animals in which the ink cleared very rapidly, blood samples were taken one, three, five, seven, nine, and ten minutes after injection. The samples were immediately hemolyzed in 2 ml. of a 1:1,000 dilution of sodium carbonate (Na_2CO_3) in distilled water. The amount of carbon present in the sample was then determined electrophotometrically in a Coleman colorimeter set at 675 lambdas. From this reading the logarithm of the true concentration of carbon particles was calculated. Once the carbon clearance had been completed, the animals were killed with chloroform and their livers and spleens were weighed.

The following values were determined for each animal:

(*1*) Body weight

(*2*) Combined weights of liver and spleen

(*3*) The quotient: $\frac{\text{body weight}}{(\text{liver} + \text{spleen}) \text{ weight}}$

[6] The compounds were kindly supplied by Dr. Russell W. Schaedler of the Rockefeller Institute.

[7] The material used was prepared by Dr. Baruj Benacerraf of New York University Medical School from the commercially available suspension C 11 1431a (produced by Günther Wagner, Hanover, Germany), and was generously supplied by him.

(4) The clearance value k. This factor was determined by plotting the log of the carbon concentration in each blood sample against the time of bleeding. The points thus obtained give an approximately straight line. The slope of this curve is the value k, expressed by the equation:

$$k = \frac{(\log \text{conc. } 1) - (\log \text{conc. } 2)}{t_2 - t_1}$$

in which t_1 and t_2 represent the time in minutes when samples 1 and 2 were withdrawn (text figure 2A).

(5) The corrected phagocytic index α is the value k corrected for the weights of liver and spleen in relation to the body weight. This factor serves as an index of the activity of the reticuloendothelial system in relation to quotient 3, and is described by the equation:

$$\alpha = \sqrt[3]{k} \times \frac{\text{body weight}}{(\text{liver} + \text{spleen}) \text{ weight}}.$$

On each test day and for each group the arithmetic means and standard deviation were calculated for factors 2, 3, 4, and 5. Results exceeding twice the standard deviation of the control values were considered statistically significant (15).

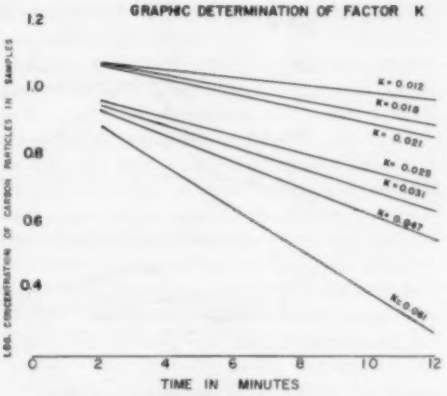
For differential counts, one drop of blood was withdrawn from the retro-orbital venous plexus before the injection of carbon, spread on a defatted microscope slide, and stained by the May-Gruenewald-Giemsa method. Two independent observers each counted 300 leukocytes under oil immersion. The arithmetic means of their counts were calculated first for each single animal and then for all of the animals in a group of five.

Each of the animals was autopsied immediately after death, and its liver, spleen, and one kidney fixed in buffered formalin; the other kidney was fixed in absolute alcohol. Lymph nodes, lungs, and peritoneal nodules were also fixed whenever it seemed indicated. The fixed organs were then embedded in paraffin, routinely sectioned at 5–7 μ, stained with hematoxylin and eosin for all of the sections and, in some cases, with the Ziehl-Neelsen method for acid-fast material, Foote's Silver stain for argentaphilic fibers, the periodic acid-Schiff Hotchkiss-McManus stain, and Mallory's modification of the Gomori stain for hemosiderin.

Results

Phagocytic Acitivity

The method used to determine the numerical value of the clearance factor k is illustrated in text figure 2A. The slope of the straight line obtained by plotting the logarithm of carbon concentration against time in minutes is represented by k. For each group, the arithmetic means of numerical values for k, α, liver and spleen weights,

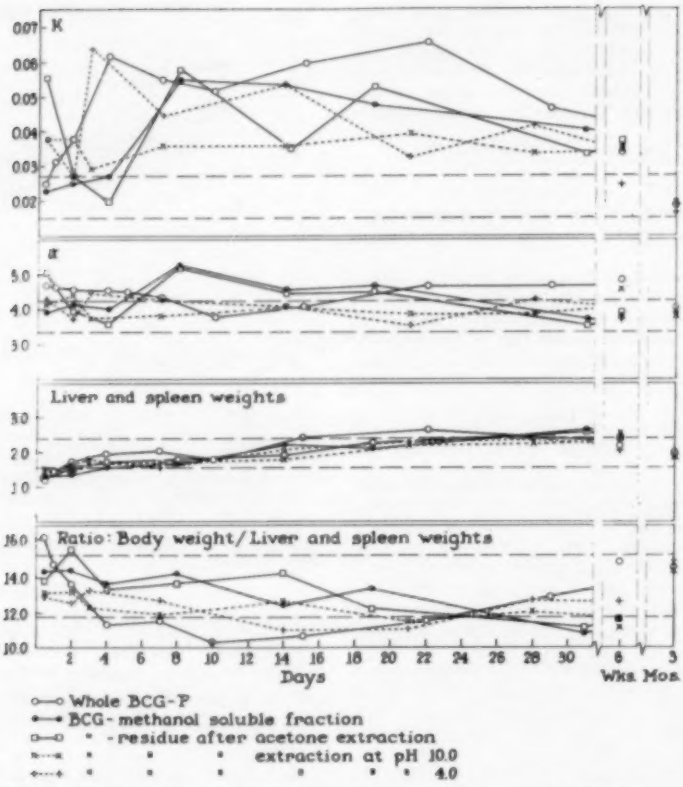


TEXT-FIG. 2A. Graphic representation of clearance rate of carbon particles, determining the numerical value k for a given group of animals tested. The slopes shown on this graph correspond to the 4 values of k plotted in text figure 2B for the fraction BCG-P on days 1, 2, 4, and 6. The lines on the upper part of the graph are those obtained for control animals.

and the ratio of body-weight/liver and spleen weight, have been calculated, plotted against time for the entire duration of the experiment, and are presented in text figure 2B. The broken lines indicate the standard deviation of the controls.

As may be seen in text figure 2B, *group 2*, which had been treated with BCG-P, responded with a steady rise in activity of the reticuloendothelial system as expressed by the clearance factor k over the first four days. This high level of activity was maintained until the third week after injection; thereafter it fell off gradually and reached control levels again by the third month. The corrected phagocytic index α, on the contrary, did not show any conspicuous changes and never rose significantly above control levels. This difference is explained by an initial drop of the ratio values caused by a marked weight gain of liver and spleen which was out of proportion to the general growth of the animal. By the third week, the original relation between these two factors had been re-established, but now the falling k values did not permit any more α to rise to a significant level.

The third group, which had received the BCG residue after acetone extraction, showed an altogether different picture. Both the global



TEXT-FIG. 2*B*. Effects of constituents of *M. tuberculosis* upon clearance rate of carbon particles. Each point corresponds to the arithmetic means of 5 animals; the broken line indicates the standard deviation of the untreated controls.

clearance activity (k) and the corrected phagocytic index α displayed a biphasic pattern of response. An immediate initial rise was quickly followed by a drop, but only to go over into a new, significant peak. The second maximum returned slowly to normal over the next four weeks. No unusual change in the liver and spleen weights was observed. They gradually increased with age in relation to the body weight, which accounts for the level course of the ratio curve.

In *group 4*, which had been treated with methanol extract, both k and α rose slowly during the first week—k even longer—and a high degree of activity was maintained until the third week. Thereafter, a gradual fall was noted and, by the fifth week, no significant difference could be observed any longer between the treated and the untreated animals. As in the previous group, neither liver nor spleen showed any accelerated weight gain. Their growth curves remained in proportion to the increase in body weight generally observed with age.

It must be mentioned here that *groups 3* and *4* were followed only for a period of six weeks.

The injection of preparation "pH 10" evoked only a fairly weak response in *group 5*. The activity as expressed by factor k rose immediately to the borderline of statistical significance and maintained a constant level for practically six weeks, whereafter it dropped to control values. The level course of this curve is all the more striking if the very small standard deviation uniformly observed for all samples is taken into consideration. Neither the ratio nor the corrected phagocytic index showed any conspicuous changes.

Group 6, which had received treatment with preparation "pH 4.0," presented a pattern of reaction quite different from that of *group 5*. The animals responded with a sharp rise of their ability to clear injected carbon from their circulation. Factor *k* reached a maximum on the fourth day and remained significantly elevated for a period of two weeks, whereafter it gradually returned to normal. The corrected phagocytic index, however, behaved similarly to the value obtained in *group 5*, i. e., never rose above control limits. This fact had to be interpreted as a consequence of a slow, progressive drop in ratio values which, although it never reached statistical significance, prevented α from paralleling the *k* variations.

Circulating Leukocytes

In the conditions of the experiment, the changes recorded in the percentage distribution of circulating leukocytes were very slight and of no statistical significance when compared with the figures obtained from the control groups; therefore, no graph is presented for those values. Only in *group 2*, injected with acetone-extracted BCG, was there a moderate increase of segmented neutrophils noticed between the first and the fourth days. Besides, a transient increase of the monocyte/lymphocyte ratio was observed on the eighth day. The average figure for this group was then 0.520 (26/50), against 0.139 (10/72) for the first day, and 0.169 (11/65) to 0.350 (20/57) as the arithmetic over-all means for the control animals.

Anatomic Changes

It was to be expected that compounds so closely related to each other as those tested in the present experiment would cause, if any, tissue modifications of the same kind. The findings were, in fact, very much the same for the different groups at given times, so that it seems reasonable to give a general account of the changes observed. In brief, following a local and general inflammatory reaction which had no specific character, a widespread histiocytosis developed together with multicentric heterotopic hematopoiesis. The latter regressed at the time (two to three weeks) when histioreticular cells coalesced and underwent epithelioid changes, thus resulting in a generalized nodular reticulosis which, according to the definition given thirty-two years ago by Sabin, Doan, and Forkner (9), was indeed "tubercular."

Macroscopic examination: At autopsy, macroscopic inspection disclosed the presence, in the early stages, of a localized peritoneal reaction consisting of yellowish, infiltrated, soft nodules attached to the mesenteries and omentum. A frequent location of these nodes was the left colic angle. In the case of the methanol extract, only discrete exudates were found and the appearance of localized granulomas was delayed. The Peyer's patches were enlarged and granular. In later stages the nodes became, as a rule, larger and fibrotic and formed numerous adhesions with the neighboring structures. No conspicuous alterations were noticed in livers, spleens, and kidneys.

Microscopic examination: The most striking changes after twelve and forty-eight hours were localized in the peritoneal cavity. A strong inflammatory reaction was present, particularly in the case of less soluble materials, which could generally be identified in the center of the exudate. In the sections stained with hematoxylin and eosin those materials were faintly basophilic. When whole killed bacilli (BCG-P) had been given, most of them remained acid-fast for several days. The particles, as well as the bacilli were usually agglutinated and surrounded by polynuclear cells and fibrin. The most irritating fractions were pH 10, pH 4, and BCG-residue. In these groups the aggregates of hypersegmented granulocytes were particularly dense, the formation of a pyogenic membrane could be observed, and necrotic areas appeared.

At the periphery of these foci, histiocytes and fibroblasts were readily observed at forty-eight hours and multiplication of mesenchymal cells was active. Vessels in the neighborhood showed stasis, leukocytic margination, and perivascular edema. The endothelial cells of many capillaries and arterioles were swollen, and some of them had retained carbon in considerable amount. As a rule the carbon uptake was always found increased in inflammatory areas. In the omental fatty tissue, lymphostasis and plasmostasis, opening of Doan's "dormant" capillaries, and infiltration by lymphocytic cells occurred. In most cases the pancreatic tissue was involved in the acute process and was heavily infiltrated, but the Langerhans' islets remained morphologically intact throughout the survey, and hemorrhagic pancreatitis did not develop.

Signs of generalized inflammation were found at that time in the kidneys and liver. In the liver, the Kupffer cells appeared swollen and had very prominent nuclei in the animals injected with carbon as well as in those sacrificed at the same time without injection. In the former the carbon distribution among the stellate cells was very even. The parenchymatous cells looked normal in all groups, and necroses were never recorded. In the lymphoid organs, so-called secondary centers were numerous and were surrounded by a ring of small, mature lymphocytes. "Reaction centers" with mitoses, intercellular edema, chromatinian bodies, and cells with extremely large vesicular nuclei were observed throughout the entire experiment. In the lymph nodes, heavy sinus catarrh developed while, in the spleen, stasis, accumulation of hypersegmented pyknotic neutrophils, and swelling of the endothelial cells lining the sinuses were frequently noted.

The writers have mentioned elsewhere (8) the presence in normal mice of a specially developed lymphoreticular tissue in the perivascular space of the portal area in the liver and in the interarteriovenous connective net of the kidney. This tissue seems to take part in a great number of systemic reactions, and was found to proliferate very early after the administration of the bacillary extracts. This reaction never presented a "specific" pattern.

As early as forty-eight hours after injection and in all treated groups, two important phenomena took place: First, numerous interstitial foci of histiocytic proliferation appeared in the liver while the histiomonocytic population in the peritoneal exudates increased. Second, hepatic hematopoiesis became conspicuous, particularly in animals injected with BCG-P, as is shown in figure 1. Young megakaryocytes were numerous, and obviously not embolic. Later on, foci of hematopoiesis could be seen in most of the lymph nodes examined (figure 2). Since splenic myelopoiesis is a normal feature in adult mice, it was difficult to draw any quantitative estimate as to whether it was more active in treated animals. Nonetheless, wide fields of myelo- and promyelocytes were more often observed in animals given BCG fractions than in normal controls. Careful search for erythrophagic activity in the sinuses brought negative results. Throughout the entire survey, iron-staining material was not increased in the spleens of treated animals when compared with controls. The bone marrow was not examined.

During the weeks following injection, the granulomas which had formed in the peritoneal cavity exhibited a characteristic picture (figure 3). They were walled off by several layers of young and mature fibrocytes, but in their central portion new necrotic foci continuously appeared, first surrounded by polynuclears and, later, by histiocytes. The latter became by far the prominent cell type in the granulation tissue, they acquired a definite epithelioid character by the third week, and converged to form small concentric nodes (figure 4) in which deposition of argentaphilic fibrils soon took place. Giant cells of the Langhans' type were never observed. In the most advanced stage the typical lesion could be described as a *chronic epithelioid granulomatosis*. Isolated or confluent nodes were likewise found scattered on the mesenteric serosa, around the pancreas, in the lymph nodes, in the liver (figure 5), and in the spleen (figure 6). In this organ, not only focal epithelioid but generalized reticulosis occurred, resulting in a marked increase of interstitial tissue in the red pulp and in a concomitant diminution of hematopoietic fields; while, at times, a suggestive augmentation of argentaphilic and collagenic fibers was noticed around the centrofollicular arteries. Thus, in those cases, a progressive "fibroadenia" indeed developed, while formation of new centers progressed in a budlike fashion around distal arterial branches. As to the injected carbon, splenic uptake uniformly appeared to be diminished in treated animals.

The histiocytic granulomas of the liver followed the general trend of epithelioid transformation, but remained isolated. Neither diffuse interstitial fibrosis nor portal cirrhosis were ever observed. In some cases the small tubercles seemed to disappear without scars. With the exception of the central portion of the peritoneal granulomas, where recurrent necrosis was found up to the third month of observation, the productive character of the epithelioid structures was extremely consistent, and caseation never occurred. All throughout the survey, the kidneys remained remarkably unchanged with the exception of the transient inflammatory reaction observed during the early phase.

It is worth mentioning that the injected

material as such could never be identified in lymphatic channels or in lymph nodes, nor in any location other than the peritoneal granulomas.

Discussion

All of the compounds investigated in this study possess protective activity against a subsequent challenge with unrelated infective microorganisms (3, 7). From the results presented above, it seems that they all behave quite similarly with regard to the parameters examined.

All of them stimulated the ability of the albino mouse to clear injected carbon from the blood stream to the same extent and for the same length of time. Nothing can be said about their mode of action, and it remains an open question whether this result was achieved by increasing the carbon uptake of already active cells, by "opening up" other cells which were present but yet inactive, or by inducing the formation of new cells. Nonetheless, a pharmacologic difference between the compounds may be indicated by the fact that the variations for α followed those of k in only two groups, suggesting a functional increase in activity rather than a consequence of mere weight uptake of organs or weight loss of the animal.

The only histologic evidence of cell multiplication concerned those participating in the formation of "tubercular" structures; there was nothing to indicate that such cells, particularly when epithelioid, took any part in the carbon clearance. It should be remembered at this point that Biozzi and colleagues (6) found that granulopectic activity was diminished in BCG- and tuberculosis-infected animals at precisely the time when specific granulomas such as those observed in the present experiments appeared in great numbers.

With the exception of the BCG-acetone extract, none of the materials under study had any marked effect upon the percentage distribution of circulating leukocytes. No "showers" of monocytes could be observed in peripheral blood even at a time when the monocytic reaction in the tissues was at its peak. This fails to confirm an observation made by Sabin, Doan, and Forkner (9) in rabbits and guinea pigs and, more recently, by one of the present authors (10) in mice; but this difference might be explained by the fact that considerably larger amounts of active substances were employed by these investigators.

As to the tissue reaction induced by the different fractions, it proved to be very much the same in all of the groups observed, and definitely simulated that produced by living tubercle bacilli (16). All fractions investigated in the present study induced not only a proliferation of histioreticular tissue, but also the transformation of histiocytes into epithelioid cells and the formation of tubercles. This observation would be of little significance if such reactions had been limited to the peritoneal tissue, for foreign body granulomas and lipogranulomatosis may show epithelioid cells. But, curiously enough, neither foreign body reaction nor any of the aspects typical for steatonecrosis was ever demonstrated around the injected material. Furthermore, the fact that epithelioid granulomas were encountered in the liver, spleen, and lymph nodes favors the assumption that they indeed constitute a response to the constituents of the tubercle bacillus. In this connection it is interesting to remember that both Sabin and colleagues (9) and White (17) obtained similar histologic modifications by injecting Anderson's fractions and purified D-wax. Likewise, the similarity between the reactions obtained in the present experiments and those described after inoculation of living BCG by Calmette in rabbits (18) and, very recently, by Camain, Senecal, and Couturier (19) in the livers of vaccinated African babies, is particularly striking.

According to the present observations, it appears that the mouse does not form symplasts of the Langhans' type, although this animal is perfectly capable of producing foreign-body giant cells, for instance, around a thread of cotton. It is surprising in this regard that the bacillary material as such did not even elicit formation of giant cells of the latter type. Caseation and liquefaction were never observed.

The lymphoid tissue in the mouse exhibits certain peculiarities which have already attracted the attention of previous writers (20, 21). It seems that the lymphopoietic potentialities of the perivascular mesenchyma are greater in the mouse than in other mammals, at least in the liver and kidney. In the experiments reported, these perivascular aggregates did not take a part equal to that of the spleen and lymph nodes in the response to the injected material,

and their reaction was always nonspecific in character.

An interesting phenomenon is the persistence, in normal adult mice, of a very active splenic hematopoiesis. In addition, the liver and lymph nodes have a wide hematopoietic potentiality. This is easily demonstrated in suckling and anemic adult animals (22). Hepatic foci are usually found in intralobular capillaries, but at times can also be noticed in the periportal lymphoreticular tissue. Megakaryocytes are seen either within hematopoietic areas or are isolated (23). Under normal conditions they are known to disappear from the liver swiftly after birth, while they remain present in the spleen throughout the entire lifespan (23, 24). In the present investigation, heterotopic hematopoiesis was frequently encountered, and megakaryocytes were very often present in the liver and lymph nodes. Release of platelets was never demonstrated; megakaryocytic phagocytosis, which had been reported by Andrew (25), was observed in only two instances.

The recent suggestion that lipopolysaccharides such as dextran (26) and BCG extracts (27) would result in "hypersplenism" is not supported by the present observations. It is true that certain aspects of the spleen recall the "fibroadenia" of ancient writers, but neither erythrophagia nor pigment deposition could be demonstrated, and the very active extramedullary hematopoiesis speaks, rather, in favor of a bone-marrow dysfunction. That anemia occurs as a result of mycobacterial infection is a well-documented fact, but a determination of hemoglobin levels and the erythrocyte count were not included in the study, and only indirect evidence of anemia was established.

The aim of the present study was clearly to determine whether any correlation could be established between the various biologic changes induced by mycobacterial fractions in laboratory animals, i. e., (to name only a few) increased nonspecific resistance, increased ability to clear foreign particles introduced into the blood stream, local and general tissue reactions, and modifications of the circulating erythrocytes.

It must be recognized that under the conditions of these experiments such a correlation—which would have thrown some light on the mechanisms by which natural resistance increases following the injection of the compounds mentioned above—could not be demonstrated. The fact that an experimental anemia results in a marked stimulation of the cellular defense system of the mouse, as demonstrated by Berry and colleagues (28, 29), can certainly not be ignored at this point.

It is worth mentioning that no spontaneous death or visible tumorous growth occurred during the entire duration of the experiments.

Summary

Male Rockefeller albino mice received, by intraperitoneal injection, a single small dose of killed BCG and several subfractions derived from it. The animals uniformly responded with an increase of ability to clear injected carbon from their circulation. The compounds tested failed to have any effect upon the percentage distribution of leukocytes in the peripheral circulation.

All investigated materials induced the production of epithelioid granulomas at the site of injection, in the liver and, to a lesser extent, in the spleen and lymph nodes. This reaction was of the type observed after infection with living tubercle bacilli. Caseation of these "tubercular" lesions was never encountered.

Sumario

Efectos del Bacilo Tuberculoso (BCG) Matado y de Sus Componentes sobre el Aparato Reticuloendotelial, las Visceras y la Distribución de Leucocitos Circulantes en Ratones Albinos Normales

Ratones albinos Rockefeller machos recibieron, por inyección intraperitoneal, una pequeña dosis única de BCG muertos y de varias subfracciones derivadas de éstos. Los animales respondieron uniformemente con un aumento de su capacidad para eliminar el carbón inyectado de su circulación. Los compuestos ensayados no ejercieron efecto alguno sobre la distribución porcentaria de los leucocitos en la circulación periférica.

Todas las substancias investigadas provocaron la producción de granulomas epitelioideos en el sitio de la inyección, en el hígado y, en menor grado, en el bazo y los ganglios linfáticos. Esta reacción fué de la forma observada después de la infección con bacilos tuberculosos vivos. Jamás se observó caseación de estas lesiones "tuberculosas."

Resume

Modifications fonctionnelles et histologiques du système réticuloendothélial et de la formule leucocytaire de la souris après injection de BCG tué et de ses constituants

De jeunes souris adultes, de race albinos (souche Rockefeller) reçoivent en une seule injection intra-

péritonéale 200 γ de BCG tué ou de diverses fractions de ce bacille. Chez tous ces animaux on observe une élévation marquée et durable de l'index granulopectique pour le carbone. La formule leucocytaire n'a pas présenté de variations significatives. Toutes les fractions étudiées dans le présent travail déterminent l'apparition de granulomes épithéloïdes au lieu d'injection, dans le foie, et—à un moindre degré—dans la rate et les ganglions lymphatiques. Cette réaction est en tous points semblable à celle que provoque l'injection de bacilles tuberculeux vivants, mais leur caractère productif est particulièrement marqué et l'on n'observe jamais de caséification.

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Fig. 1. BCG-P, forty-eight hours. Liver: heterotopic, intracapillary focus of hematopoiesis. In the right part, large doughnut-shaped myelocytes and promyelocytes; on the left, nucleated erythrocytes. (May-Grunewald-Giemsa stain, × 924)

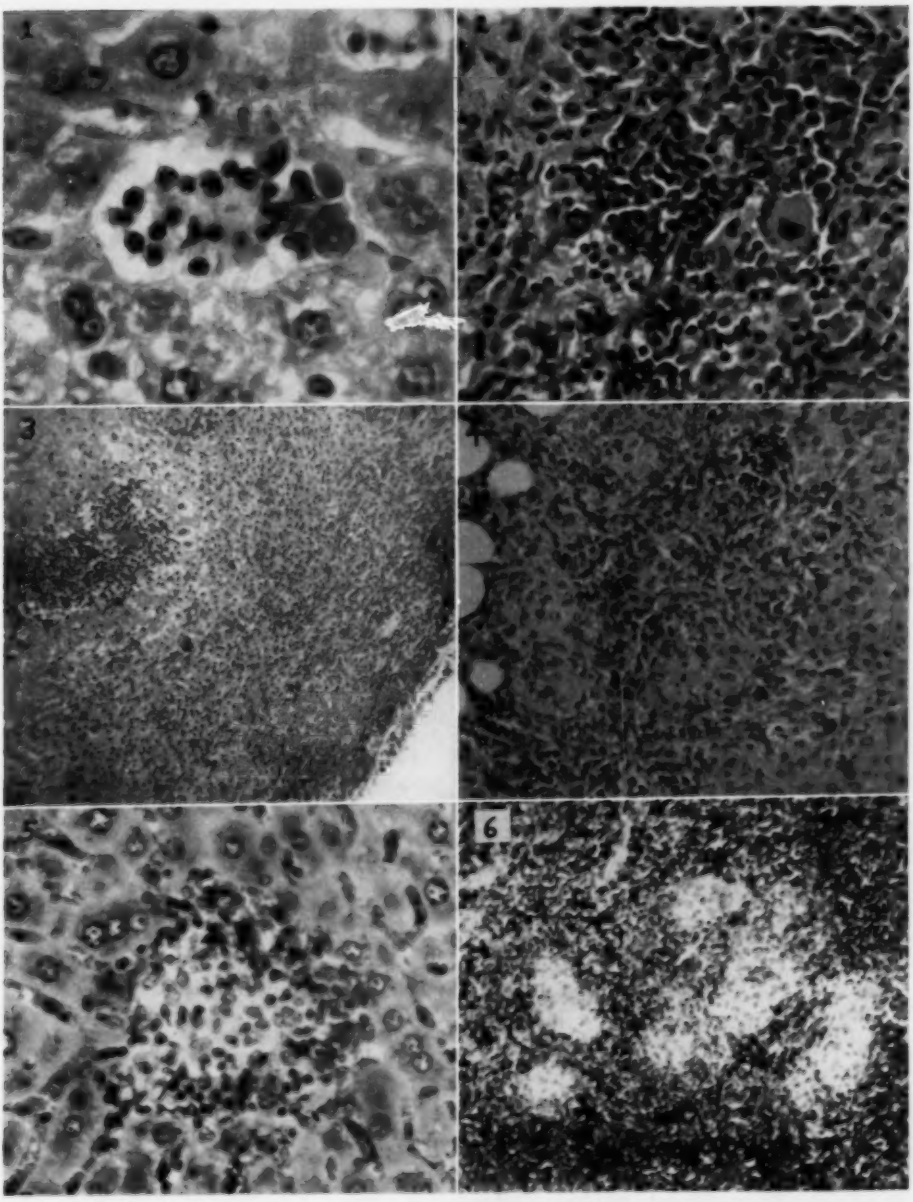
Fig. 2. BCG-pH 10, three months. Mesenteric lymph nodes. Focus of heterotopic hematopoiesis, with a megakaryocyte in the middle. (Hematoxylin and eosin, × 570)

Fig. 3. BCG-pH 10, three weeks. Peritoneal abscess with central necrosis, and numerous polymorphonuclears around the injected material. Monocytic cells at the periphery show early epithelioid changes. (Hematoxylin and eosin, × 140)

Fig. 4. BCG-pH 4, six weeks. Omental abscess: granulomatosis with numerous epithelioid cells. (Hematoxylin and eosin, × 570)

Fig. 5. BCG-P, six weeks. Liver: epithelioid granuloma. (Hematoxylin and eosin, × 570)

Fig. 6. BCG-residue, three months. Spleen: numerous tubercles with characteristic epithelioid cells. (Hematoxylin and eosin, × 250)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THE DIAGNOSTIC RELIABILITY OF ACID-FAST BACILLI DEMONSTRATED IN ASPIRATED GASTRIC CONTENTS[1]

PHILIP C. PRATT AND ROBERT J. ATWELL

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INTRODUCTION

One of the more firmly established tenets in the field of tuberculosis bacteriology is the principle that it is useless to examine smears of aspirated gastric contents for acid-fast bacilli because of the frequency of false positive results (1–5). However, there is surprisingly little published evidence pertinent to this specific point. In a paper concerned with the finding of tubercle bacilli by culture of gastric aspirates in persons with normal chest roentgenograms, Medlar, Ordway, and Pesquera (6) stated that they had not done smear studies because a previous study by Roper and Ordway (7) had shown false positive results. A review of the latter paper shows that 983 gastric smears had been performed and 28 of these showed acid-fast bacilli morphologically typical of *M. tuberculosis*. Of the 28 specimens, 19 were from known tuberculous patients and 9 were from patients believed not to have tuberculosis. Thus, their data show only 9 false positive smears in 983 tests, or about 1 per cent. On the other hand, Stiehm (8, 9) advocated the use of smears of gastric aspirations for purposes of early diagnosis, to be followed by the cultural results. In some 30 patients he had no instance in which a gastric smear was positive, but the culture was negative. Other writers (10, 11) have also presented evidence that it is rare to find acid-fast bacilli in gastric aspirations of nontuberculous patients.

During a recent study of gastric aspirations from healthy hospital personnel (12), the writers noted that the incidence of acid-fast bacilli demonstrated by microscopy in such a group was only 11 in 285 specimens. In no instance were two positive specimens obtained from a single individual. In view of these findings, and with the knowledge that some clinicians (13) do use gastric smears for early diagnosis of tuberculosis in patients without sputum, the present study has been done to evaluate the reliability of this procedure.

METHOD

Throughout the period of this study, gastric aspirations were done according to an "improved technique" described previously (12). In essence, this technique involves the elimination of likely sources of contaminating organisms, such as tap water, ice, and lubricant. All specimens were treated by standard concentrating procedures involving shaking of the specimen for twenty minutes in the presence of an equal volume of 4 per cent sodium hydroxide, neutralization, and centrifugation. The smears prepared from each sediment were stained with Ziehl Neelsen stain and examined for a total of ten minutes. Specimens were reported positive on microscopy if four or more acid-fast bacilli were seen. No attempt was made to distinguish different types of organisms on the basis of morphology. Two tubes of Löwenstein's medium were inoculated from each specimen.

RESULTS

The results are presented in table 1 which includes microscopy and culture results for gastric specimens obtained from 215 patients admitted consecutively to this hospital. Of the entire group, 181 were considered to have pulmonary tuberculosis either on the basis of findings in this hospital or reports of positive cultures elsewhere; 28 were proved to have nontuberculous disease, and 6 had no diagnosis established. Among the tuberculous patients, 738 smears were examined and 55 or 7.5 per cent of these were positive for tubercle bacilli. Among the 34 nontuberculous or undiagnosed cases, 114 smears were performed and none were positive. The remaining entries in the table give details of the correlation between gastric smear and culture results. It may be noted that 44 of the 55 specimens positive on smear were also positive on culture. The remaining 11 "false positive" smears deserve attention.

The "false positive" findings occurred in specimens from 5 different persons, all of whom had been on long-term therapy for pulmonary

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TABLE 1

TABULATION OF DATA CONCERNING INCIDENCE OF SMEARS POSITIVE FOR ACID-FAST BACILLI AND CORRELATION WITH CULTURE RESULTS IN GASTRIC ASPIRATIONS AND SPUTUM

Item	Gastric Washings			Sputum		
Diagnosis	Tuberculosis	Nontuberculous Disease	Unknown	Tuberculosis	Nontuberculous Disease	Unknown
Number of persons	181	28	6	229	28	5
Number of smears	738	97	17	1,776	101	21
Number of smears positive for acid-fast bacilli	55	0	0	535	0	0
Per cent of positive smears	7.5	0	0	3.0	0	0
Number of persons with positive smears	25	0	0	152	0	0
Per cent of persons with positive smears	13.8	0	0	65.4	0	0
Smear positive, culture positive for *M. tuberculosis*	44 (19)*	0	0	303 (80)	0	0
Smear positive, culture negative for *M. tuberlosis*	11 (5)	0	0	232 (71)	0	0
Smear negative, culture positive for *M. tuberlosis*	66 (35)	0	0	62 (32)	0	0
Smear positive, culture positive for unclassified acid-fast bacilli	1 (1)	0	0	1 (1)	0	0
Smear negative, culture positive for unclassified acid-fast bacilli	15 (14)	3	1	9 (8)	0	1 (1)

* Figures in ( ) indicate number of patients from whom the specimens were obtained.

tuberculosis, the diagnosis having been established by multiple cultures of *Mycobacterium tuberculosis*. One patient who had three "false positive" gastric smears also produced seven sputum specimens with positive smears and negative cultures. Another patient had three such gastric specimens on consecutive days occurring two months after "conversion" of cultures from positive to negative. The third and fourth patients had been treated for twelve and seven months and had three and one isolated "false positive" smear reports, respectively. The fifth instance occurred in a patient who had been on treatment for six months. The organisms in this case were filamentous in character but were included because they were acid fast.

The table also shows comparable data for sputum findings during part of the period covered by microscopy of gastric washings.

DISCUSSION

The findings reported here indicate strongly that smears of gastric aspirations are much more useful for the purpose of early diagnosis of tuberculosis than has been generally believed heretofore. In fact, such preparations appear to be just as reliable as sputum smears. The widespread assumption that acid-fast bacilli from food, teeth, and other sources would occur so frequently as to obviate the usefulness of gastric smears is not borne out by this study in hospital patients with pulmonary disease.

That the examination of gastric smears can be useful diagnostically is revealed by the fact that the organisms were found by this means in the absence of sputum in 12 of the 215 patients. These patients would otherwise have had to be started on treatment empirically or would have had to await the results of cultures of their specimens. Needless to say, the final identification of *M. tuberculosis* depends on cultural morphology and other factors, whether found in gastric contents or sputum.

The fact that acid-fast bacilli other than tubercle bacilli are only rarely found in gastric smears is probably a reflection of the fact that such organisms when present are extremely few in number. Thus it has been noted (14) that the unclassified bacteria in cultures usually occur as only one or two colonies unless the patient actually has infection caused by these organisms.

The 11 "false positive" smears among 852 specimens examined all occurred in patients already on treatment for proved tuberculosis

for six or more months. It is well known that patients on treatment may at times excrete acid-fast bacilli which do not grow in culture. This is also a common finding in resected lesions after six to eight months of treatment. Therefore, it is likely that most of the instances of "false positive" gastric smears encountered in this study can be explained on this basis.

Summary

A series of specimens obtained by gastric aspiration from patients in a tuberculosis hospital has been examined for acid-fast bacilli by smear and culture.

Most specimens which were positive for acid-fast bacilli on microscopy were also positive on culture; a few were negative on culture. The latter specimens were obtained from patients already being treated for proved tuberculosis. It may be concluded that the demonstration of acid-fast bacilli by microscopy of gastric aspirations from patients suspected of having pulmonary tuberculosis is as reliable for purposes of early diagnosis as demonstration in the sputum. Acid-fast bacilli other than *M. tuberculosis* were rarely found in smears of gastric aspirations. The smears of specimens from which unclassified mycobacteria were cultured were negative in all but one instance.

Sumario

Credibilidad Diagnóstica de los Bacilos Acidorresistentes Descubiertos en el Contenido Gástrico Aspirado

Una serie de ejemplares obtenidos por la aspiración gástrica de enfermos de un hospital para tuberculosos ha sido estudiada en busca de bacilos ácidorresistentes en frotes y cultivos.

La mayor parte de los ejemplares que fueron positivos microscópicamente para los bacilos ácidorresistentes también lo fueron en los cultivos; algunos fueron negativos en éstos. Los últimos procedían de enfermos ya en vías de tratamiento por tuberculosis comprobada. Cabe deducir que el descubrimiento por la microscopía de bacilos ácidorresistentes en las aspiraciones gástricas de enfermos en que se sospecha tuberculosis pulmonar es tan fidedigno para los fines del diagnóstico temprano como el descubrimiento en el esputo. Rara vez se encontraron bacilos ácidorresistentes distintos del *M. tuberculosis* en los frotes de las aspiraciones gástricas. Los frotes de los ejemplares de los que se cultivaron micobacterias inclasificadas fueron negativos en todos los casos, menos uno.

Resume

La sureté du diagnostic de bacilles acido-résistants mis en évidence dans des contenus gastriques aspirés

Une série d'échantillons obtenus par aspiration gastrique de malades dans un hôpital pour tuberculeux a été examinée à la recherche de bacilles acido-résistants, au moyen de lames et de cultures.

La plupart des échantillons qui furent, à l'examen microscopique, positifs pour les bacilles acido-résistants le furent aussi dan. .s cultures; quelques uns furent négatifs dans les cultures. Ces derniers échantillons furent obtenus chez des malades ayant déjà été soignés pour une tuberculose avérée. On peut conclure que la mise en évidence de bacilles acido-résistants au moyen d'examen microscopique d'aspirations gastriques de malades soupçonnés de tuberculose pulmonaire est aussi sure pour établir un diagnostic précoce qu'une mise en évidence dans l'expectoration. Des bacilles acido-résistants autres que *M. tuberculosis* furent rarement trouvés dans les lames d'aspirations gastriques. Les plaques d'échantillons à partir desquels des mycobactéries non classées furent cultivées furent négatives chez tous sauf un.

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STUDIES ON MYCOBACTERIUM TUBERCULOSIS H37RV GROWN IN VIVO[1]

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INTRODUCTION

Studies on pathogenic bacteria in general and on *Mycobacterium tuberculosis* in particular were made in a majority of cases on bacteria grown *in vitro* under conditions different from those prevailing in the host. The study of tubercle bacilli grown *in vivo* may shed some light on factors and processes responsible for their pathogenicity. There are several reports dealing with the differences between tubercle bacilli grown *in vivo* and *in vitro*.

Sheehan and Whitwell (1) described a difference in fat staining between organisms grown *in vivo* and *in vitro*. Bekierkunst and associates (2) noted that tubercle bacilli from pathologic material required less glycerol for growth than laboratory cultures. Segal and Bloch (3) showed that the addition of various substrates had no stimulating effect on the endogenous respiration of the tubercle bacilli grown *in vivo*, although the same substrates brought about a large increase in respiration of the cells grown *in vitro*. Those writers also suggested a difference in the surface structure between the two kinds of organisms, by virtue of the fact that the organisms grown *in vivo* gave stable and uniform suspensions in water or saline, whereas those grown *in vitro* did so only when Tween® 80 was present in the growth medium. Since the lack of response to glucose and its intermediates could not be explained by permeability barrier or saturation of the corresponding enzymes with the aforementioned substrates (other substrates, salicylic acid for example, did elicit a positive response) (3), the question arose as to what substrate was being used by tubercle bacilli grown *in vivo* to satisfy their energy requirements. An approach to this problem was made by the present writers by studying the respiration of the cell-free extracts of the bacilli which, if enzymically active, could be freed of the endogenous substrates by dialysis. However, the studies have shown that cell-free extracts of *Mycobacterium tuberculosis* grown *in vivo* were completely devoid of dehydrogenase activity and the capacity to respire. In a preliminary note (4) it was reported that this was due to an "inhibitor" present in their extracts.

This paper deals with further investigations on the inhibition displayed by the tubercle bacilli grown *in vivo*.

MATERIAL AND METHODS

Organisms and growth media: The organisms used were: *Mycobacterium tuberculosis* H37Rv, *Mycobacterium tuberculosis* BCG, and *Mycobacterium phlei*. The strains BCG and H37Rv were grown and maintained in a liquid medium of the following composition: asparagine, 5.0 gm.; KH_2PO_4, 5.0 gm.; citric acid, 1.5 gm.; ferric ammonium citrate, 0.05 gm.; $MgSO_4$, 0.5 gm.; K_2SO_4, 0.5 gm.; glycerol, 0.5 gm.; Tween 80, 0.5 gm.; distilled water to 1,000 ml. pH was adjusted to 6.8. Ten per cent bovine albumin was added after sterilization to a final concentration of 0.25 per cent.

The cells were grown without shaking at 37°C. Ten-day-old cultures were used unless otherwise stated. *M. phlei* was grown at 37°C. on a rotary shaker in a Difco nutrient broth supplemented with 0.5 per cent Tween 80, sodium chloride, 0.5 per cent, and K_2HPO_4, 0.25 per cent. Three-day-old cultures were used.

The tubercle bacilli grown in *vivo* (LH37Rv) were obtained from lungs of moribund mice infected with *M. tuberculosis* H37Rv. For the preparation of cell-free extracts of LH37Rv in sufficient quantities, at least 16 mice were required. If all the required lungs could not be obtained on the same day, they were collected and stored at −15°C. Preliminary experiments showed no difference in the behavior of LH37Rv extracts prepared from either fresh or stored lungs.

White mice, weighing 15 to 18 gm., were infected intravenously with 0.2 ml. of a six-day-old culture of H37Rv in the Tween 80 albumin medium or with a suspension of H37Rv grown on a slope of Löwenstein medium. The mice usually died at the end of the third week. Some batches of mice received intramuscular injections of cortisone acetate (0.1 mg.) every second day, starting from the fourteenth day after infection. This treatment enhanced the mortality of mice and increased the yield of tubercle bacilli.

Preparation of nonproliferating suspensions (intact cells): The cells of BCG, H37Rv, and *M. phlei* were separated from the medium by centrifugation in a MSE high-speed centrifuge at

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9,000 r.p.m. for five minutes and washed twice in a 0.1M phosphate buffer pH 7.2 containing 0.05 per cent Tween 80. When the intact cells of BCG or H37Rv were to be used in the presence of lung homogenates, Tween 80 was omitted from the washing solution after preliminary experiments had shown that Tween itself inhibited the respiration of the lung homogenates. After washing, the bacterial pellet was resuspended in the phosphate buffer, with or without Tween, or in distilled water according to the requirements.

The method of separation of LH37Rv from the lungs of infected mice was essentially that of Segal and Bloch (3) with slight modifications. The lungs were homogenized using distilled water (4 ml. of water for the lungs of one mouse), in a Teflon homogenizer run at top speed for two minutes. The homogenates were centrifuged in 250-ml. containers at 2,000 r.p.m. for twenty minutes. The supernatant containing light tissue components was used for the preparation of lung extracts; this was designated as supernatant S_1. The sediment comprising heavy tissue and tubercle bacilli was resuspended in a 0.1M phosphate buffer pH 7.2 to the original volume and centrifuged for five minutes at 1,500 r.p.m. The supernatant S_2 containing the tubercle bacilli was put aside. The sediment of heavy tissue and tubercle bacilli was treated twice in the same way. The sediment was discarded, and the three supernatants (S_2, S_3, and S_4) containing the tubercle bacilli were combined and centrifuged at 2,000 r.p.m. for fifteen minutes.

This operation was repeated until the smears stained by the Ziehl-Nielsen method showed acid-fast bacilli free of any decolorized material (tissue debris). It usually took four to seven washings. The last washing served as controls for the inhibition of hydrogen transfer activity. The suspensions of BCG, H37Rv, and LH37Rv were brought to the desired density, using a Coleman Jr. spectrophotometer. A standard curve relating the density to the dry weight of the bacteria was prepared, using suspensions of known dry weight. The dry weight of standard suspensions was determined by weighing the suspensions dried at 105°C. for twenty-four hours.

Preparation of acetone-dried powder of M. phlei: Thick suspensions of washed cells of *M. phlei* were poured into ice cold acetone (−10°C.) with stirring, allowed to stand for ten minutes, and centrifuged. This procedure was repeated twice. The pellet was then spread on a Petri dish and dried in a desiccator *in vacuo*. The dry powder could be stored at 4°C. for at least four weeks without any loss of enzymic activity.

Preparation of cell-free extracts of M. tuberculosis BCG, H37Rv, LH37Rv, and M. phlei: Suspensions of BCG, H37Rv, LH37Rv, and acetone-dried cells of *M. phlei* in 0.1M phosphate buffer pH 7.2 were disintegrated in a 9KC Raytheon magnetostriction oscillator. *M. phlei* was sonicated for forty-five minutes; BCG, H37Rv, and LH37Rv, for one hour. Broken cells and debris were removed by centrifugation at 9,000 r.p.m. for ten minutes in the cold. The clear yellowish cell-free supernatant was used. The protein content of the cell-free extracts was estimated by the biuret method.

Infection of mice with Klebsiella pneumoniae: Klebsiella pneumoniae, strain B5055 (5), was used. White mice were infected intranasally under slight ether narcosis with a thick suspension of *K. pneumoniae* from a twenty-four-hour culture.

Preparation of lung extracts (S_1): The supernatant containing light tissue after the first centrifugation of the infected lung homogenates was spun at 10,000 r.p.m. for ten minutes in a high-speed centrifuge. The sediment was discarded and the reddish opalescent supernatant was employed. The extracts of lungs of normal mice and of mice infected with *K. pneumoniae* were prepared in the same way.

Preparation of lung homogenates: Ten per cent lung homogenates in 0.25M sucrose were prepared according to the method described by Umbreit and co-workers (6).

Oxygen uptake was measured by the conventional method in the Warburg apparatus. Dehydrogenase activity was measured by the reduction of triphenyltetrazolium chloride under aerobic conditions. The formazan formed was extracted with *iso*-butanol and its color intensity was read in a Coleman Jr. spectrophotometer at 485 mμ. The values were expressed in optical density of formazan.

Results

Hydrogen transfer capacity of cell-free extracts of BCG, H37Rv, and LH37Rv: A series of preliminary experiments had shown that the oxygen uptake by cell-free extracts preparations of BCG, H37Rv, and LH37Rv was negligible. Therefore, their hydrogen transfer capacity,

TABLE 1

Reduction of Tetrazolium by Cell-free Extracts of M. Tuberculosis BCG, H37Rv, and LH37Rv in the Presence of Lactate and Malate

Substrate	Optical Density of Formazan Formed by		
	BCG	H37Rv	LH37Rv
Endogenous	0.00	0.00	0.00
Lactate	1.20	1.75	0.00
Malate	3.20	1.00	0.00

The reaction mixture contained: 10 mg. of extract protein; 30 μmoles of substrate; 50 μmoles of phosphate buffer pH 7.2; 1 per cent triphenyltetrazolium chloride, 0.1 ml.; final volume 1.2 ml. The reaction was arrested after one hour by the addition of *iso*-butanol.

using triphenyltetrazolium chloride as an electron acceptor was examined. The results of a large number of experiments on the reduction of tetrazolium by cell-free extracts of BCG, H37Rv, and LH37Rv are summarized in table 1. The figures are average for several preparations, but some variations were found from preparation to preparation. It is evident from the data that the cell-free extracts of BCG and H37Rv are capable of oxidizing lactate and malate in the presence of tetrazolium as an electron acceptor. However, the cell-free extracts of the tubercle bacilli grown *in vivo* (LH37Rv) are completely devoid of any activity in this respect. A great number of subsequent experiments with different batches of LH37Rv extracts in the presence of various substrates, among them glucose, glycerol, pyruvate, glutamate, glutamine, aspartate, asparagine, and α-ketoglutarate, have confirmed these results.

TABLE 2

INHIBITION OF LACTIC DEHYDROGENASE OF CELL-FREE EXTRACTS OF M. TUBERCULOSIS BCG AND H37Rv BY CELL-FREE EXTRACTS OF LH37Rv

Cell-free Extracts	Optical Density of Formazan Formed in the Presence of 30 μmoles of Lactate	Inhibition
		per cent
LH37Rv	0.00	
BCG	2.20	
H37Rv	1.46	
BCG + LH37Rv	0.24	89.0
H37Rv + LH37Rv	0.26	82.2

For experimental conditions *see* table 1.

Inhibition of lactic dehydrogenase of cell-free extracts of BCG and H37Rv by LH37Rv extracts: The complete lack of hydrogen transfer capacity of cell-free extracts of LH37Rv suggested that this might be due to the presence of an inhibitor in the extracts. This assumption was tested by the determination of lactic dehydrogenase activity of cell-free extracts of BCG and H37Rv in the presence of LH37Rv extracts. The results of these experiments, summarized in table 2, represent an average of six experiments. It may be seen from the table that the LH37Rv extracts inhibit the lactic dehydrogenase of cell-free extracts of BCG and H37Rv by 87 per cent on the average. This result prompted the testing of the effect of LH37Rv extracts on the respiration of lung homogenates.

Effect of mycobacterial extracts on succinoxidase of normal lung homogenates: Preliminary tests on the rate of respiration of lung homogenates with a number of commonly employed substrates had shown that the highest rate was obtained with succinate. Succinate was, therefore, selected as a substrate for all subsequent experiments involving the respiration of lung homogenates. The results of the experiments showing the effect of BCG, H37Rv, and LH37Rv extracts on the respiration of lung homogenates are presented in table 3. It was evident that the extracts of

TABLE 3

EFFECT OF MYCOBACTERIAL EXTRACTS ON SUCCINOXIDASE OF NORMAL MICE LUNG HOMOGENATES

Number of Experiment	Lung Homogenate μl O_2	Lung Homogenate + BCG		Lung Homogenate + H37Rv		Lung Homogenate + LH37Rv	
		μl O_2	Inhibition	μl O_2	Inhibition	μl O_2	Inhibition
			per cent		*per cent*		*per cent*
1	52	52	0.0	47	10.0	26	50.0
2	38			37	0.0	20	27.0
3	54			47	17.0	33	39.0
4	32	32	0.0	32	0.0	18	44.0
5	45			45	0.0	27	40.0
6	30	30	0.0	31	0.0	16	47.0
7	34	34	0.0	36	0.0	20	41.0
8	40			41	0.0	14	65.0
9	36	29	19.0	29	19.0	20	44.0

Each Warburg vessel contained: lung homogenates, 0.7 ml.; bacterial extracts (when present) in 0.1 M phosphate buffer pH 7.2, equivalent to 5 to 7 mg. of protein; 0.3 M succinate, 0.3 ml., tipped from the side-arm after ten minutes equilibration; 15 per cent KOH, 0.2 ml. in the center well; the final volume, 2.2 ml.; the gas phase air; temperature 37°C. Duration of the experiments, thirty minutes.

TABLE 4

EFFECT OF CELL-FREE EXTRACTS OF M. TUBERCULOSIS BCG, H37Rv, AND LH37Rv ON LACTIC DEHYDROGENASE OF CELL-FREE EXTRACTS OF M. PHLEI

Cell-free Extracts	Formazan-formed Optical Density	Inhibition
		per cent
M. phlei	1.10	
BCG	0.00	
M. phlei + BCG	1.38	—
H37Rv	0.00	
M. phlei + H37Rv	1.35	—
LH37Rv	0.00	
M. phlei + LH37Rv	0.13	88.0

The reaction mixture contained: 0.5 ml. of *M. phlei* extracts in 0.1 M phosphate buffer pH 7.2 containing 6 mg. of protein; 0.3 M lactate, 0.1 ml.; BCG, H37Rv, and LH37Rv extracts contained 3 mg. of protein; 1 per cent tetrazolium, 0.1 ml.; final volume, 1.2 ml. The reaction was stopped after ten minutes.

LH37Rv inhibited the succinoxidase of lung homogenates of normal mice from 27 to 65 per cent, the average being 45 per cent. The BCG extracts, on the other hand, were without effect on the respiration of lung homogenates. In only one experiment was a 19 per cent inhibition observed. The effect of H37Rv extracts was in general similar to that of the BCG extracts. In three of a total of nine experiments, an average inhibition of 12 per cent was noted. These experiments were performed with ten-day-old cultures. In order to test the possibility that the extracts prepared from cells grown *in vitro* may at certain stages of growth contain the inhibitor in question, extracts of BCG and H37Rv, harvested after various periods of growth (from three to twenty days), were examined for their effect on lung succinoxidase. The results showed that neither BCG nor H37Rv extracts, regardless of the age of the culture, inhibited the respiration of lung homogenates.

Inhibition of lactic dehydrogenase of M. phlei by cell-free extracts of LH37Rv: In order to study the specificity, mode of action, and nature of the inhibitor in question, a suitable "indicator system" is indispensable. Such a system should display high sensitivity toward the inhibitor and at the same time be easy and convenient to use. In the systems described above, the extracts of BCG and H37Rv and the lung homogenates fell short of these requirements. The first were strongly inhibited by the LH37Rv extracts, but their hydrogen transfer activity was low or entirely lacking. Lung homogenates suffered from the following shortcomings: their rate of respiration was low and fell off rapidly (after fifteen to twenty minutes of incubation) and, in addition, the inhibition of their respiration by the LH37Rv extracts was relatively small. It was found expedient, therefore, at this stage, to look for an indicator system which would meet the above requirements.

The experiments have shown that cell-free extracts prepared from acetone-dried cells of *M. phlei* represent an excellent indicator system by virtue of their high hydrogen transfer activity, reproducibility, and extreme sensitivity to inhibition by the LH37Rv extracts. Moreover, cells of *M. phlei* grow rapidly and can be obtained in large quantities. The acetone-dried powder prepared from them may be stored for prolonged periods without loss of enzymatic activity.

The results are shown in table 4 of a typical experiment on the effect of BCG, H37Rv, and LH37Rv extracts on the lactic dehydrogenase of cell-free extracts of *M. phlei*. It was evident that the extracts of LH37Rv inhibited the lactic dehydrogenase of *M. phlei* by 87 per cent. BCG and H37Rv extracts did not by themselves show any dehydrogenase activity, due to their low protein content and to the short duration of the experiment. However, when added to the extracts of *M. phlei*, they caused a slight stimulation of

TABLE 5

EFFECT OF INTACT CELLS OF M. TUBERCULOSIS BCG, H37Rv, AND LH37Rv ON THE SUCCINOXIDASE OF LUNG HOMOGENATES OF NORMAL MICE AND ON LACTIC DEHYDROGENASE OF CELL-FREE EXTRACTS OF M. PHLEI

Inhibition of Lung Succinoxidase in Per Cent by Intact Cells of			Inhibition of Lactic Dehydrogenase of *M. Phlei* in Per Cent by Intact Cells of		
BCG	H37Rv	LH37Rv	BCG	H37Rv	LH37Rv
0.0	0.0	28	0.0	0.0	70.0

Inhibition of lung succinoxidase was measured in the Warburg apparatus. For the conditions *see* table 3.

Inhibition of lactic dehydrogenase of *M. phlei* extracts was determined by the reduction of triphenyltetrazolium chloride. For the conditions *see* table 1.

Six milligrams, dry weight, of intact cells were used.

TABLE 6

EFFECT OF EXTRACTS (S_1) OF NORMAL AND INFECTED LUNGS ON LACTIC DEHYDROGENASE OF CELL-FREE EXTRACTS OF M. PHLEI

Additions	Formazan Optical Density	Inhibition
		per cent
None (control)	1.80	
Extracts of lungs of normal mice	2.85	Stimulation
Extracts of lungs of mice infected with H37Rv	0.40	77.8
Extracts of lungs of mice infected with *K. pneumoniae*	2.45	Stimulation

The reaction mixture contained: extracts of *M. phlei* in 0.1 M phosphate buffer pH 7.2, containing 13 mg. of protein per 1 ml., 0.5 ml.; 0.3 M lactate 0.1 ml.; extracts of lungs when present, 0.25 ml.; tetrazolium 1.0 per cent, 0.1 ml.; final volume 1.2 ml. Reaction was stopped after ten minutes.

the dehydrogenase activity of the latter. This was undoubtedly due to the contribution of the corresponding coenzymes.

Inhibition of lactic dehydrogenase of cell-free extracts of M. phlei and of succinoxidase of lung homogenates by intact cells of LH37Rv: The experiments described so far showed the presence of an inhibitor in *M. tuberculosis* grown *in vivo*, using its extracts. The reasons for starting the work with the cell-free extracts were given in the introduction to this paper. Intact cells of LH37Rv were examined for their effect on lactic dehydrogenase of *M. phlei* and succinoxidase of lung homogenates in an attempt to determine whether this inhibitory substance could be detected, i.e., whether the site of the inhibitor is on the surface of the cell. These experiments have shown (table 5) that the effect of intact cells of LH37Rv on the aforementioned systems is similar to that of their extracts. The last washing of the LH37Rv cells served as a control and did not show any inhibitory activity. The intact cells of BCG and H37Rv were likewise without any effect on the electron transfer activity on either the lung homogenates or extracts of *M. phlei*.

Effect of extracts of lungs (S_1) on lactic dehydrogenase of M. phlei: The experiments described so far were concerned with the presence of an inhibitor in tubercle bacilli from infected lungs. It was of interest to ascertain whether this inhibitory substance was also present in the infected lung tissue.

The evidence for the presence of the inhibitor in the lungs of mice infected with *M. tuberculosis* H37Rv came from experiments with lung extracts (S_1). The results of these experiments are summarized in table 6. The figures are the average of 12 experiments with different batches of lungs. It is evident from the data given in the table that the extracts of lungs of mice infected with tubercle bacilli inhibit the lactic dehydrogenase of cell-free extracts of *M. phlei* by 77.8 per cent, on the average.

On the contrary, the extracts of lungs of moribund mice infected with *K. pneumoniae* and those of normal mice increased the lactic dehydrogenase activity of *M. phlei*. The explanation of this effect is probably the same as in the case of stimulation by the BCG and H37Rv extracts.

DISCUSSION

It has been found that the tubercle bacilli grown *in vivo* and extracts of infected lungs inhibited lactic dehydrogenase activity of cell-free extracts of *M. phlei*, BCG, and H37Rv, as well as the succinoxidase of lung homogenates. No inhibitor has so far been detected in normal lungs. This fact does not preclude the possibility, however, that the normal lungs contain a certain amount of the inhibitor in question which escaped detection under the experimental conditions. This problem is presently under investigation.

The inhibition of electron transport system by *M. tuberculosis* grown *in vivo* and by the extracts of infected lungs raised the question of the origin of the inhibitor. At the present stage of the work it is not possible to say with any degree of certainty whether the inhibitor is produced by tubercle bacilli themselves or by the host tissue and is then being adsorbed on the surface of the bacilli. The finding that the intact tubercle bacilli display an inhibitory effect to the same extent as the cell-free extracts points to the surface of the bacilli as the most probable site of the inhibitor. It is tempting to speculate on the possible role of the inhibitor in the host-parasite relationship, whatever its origin.

The lack of respiratory response of the tubercle bacilli grown *in vivo* to various substrates and the difference in surface structure between the organisms grown *in vivo* and *in vitro* found by Segal and Bloch (3) may be related in some way

to the inhibitor (due to its adsorption on the surface), but this remains to be proved.

It should be recalled that the endogenous respiration of the tubercle bacilli grown *in vivo* is not impaired, being similar to that of cells grown *in vitro* (3). Disruption of the LH37Rv cells, however, brings the inhibitor into close contact with the respiratory enzymes of the cell, resulting in a complete disappearance of its dehydrogenase activity. This seems to indicate that the inhibitor has no access to the site of the respiratory enzymes in the intact cell of the tubercle bacillus grown *in vivo*.

The fact that the inhibition of lung respiration is less pronounced than that of the dehydrogenase of cell-free extracts of *M. phlei* may be due to permeability barriers in intact mitochondria. It is not yet known how the inhibitor would affect intact cells of *M. phlei* and undamaged lung. Experiments designed to test this aspect are now in progress. Lack of inhibition, however, would by no means prove the inability of the inhibitor to penetrate the cell, since it may be adsorbed on some large particle (part of the surface structure of the bacillus), thus rendering it impermeable. Clearly, negative results will call for purification of the inhibitor in question and its detachment from the particle on which it may be adsorbed. The purification of the inhibitor and the question of its origin as well as its physical and chemical properties are presently under study.

The finding that the lungs of mice infected with *K. pneumoniae* are devoid of the inhibitory activity can by no means serve as a final proof that the inhibitor is specific for the tuberculous process. The Klebsiella infection is characterized, contrary to its characterization in tuberculosis, by its acute course and rapidly fatal outcome. To reach a final conclusion in this question, lungs or possibly other organs of mice suffering from chronic infection should be investigated for the inhibitory activity.

The question whether the inhibitor is specific for the electron transport system or is of a more general nonspecific nature, for example, the destruction of enzymic activity by the action of proteinase, et cetera, remains still to be elucidated.

Differences in electron transfer activity between normal and infected tissues have been observed by several writers. Polymorphonuclear leukocytes and liver slices from guinea pigs infected with tubercle bacilli had a higher rate of respiration than cells from normal animals (7). Martin and associates (8) found a decrease in succinic dehydrogenase activity in kidneys of guinea pigs infected with tuberculosis. A lower activity of succinic dehydrogenase of liver homogenates of mice upon injection of cord factor and virulent tubercle bacilli was reported by Kato and co-workers (9, 10). In both of these cases the lowering of dehydrogenase activity was caused by the loss or lack of a soluble factor. The present experiments, on the other hand, showed lowering of respiratory activity of lung homogenates of normal mice by an inhibitor present in lungs of mice infected with tubercle bacilli. These different findings are difficult to reconcile.

Summary

Cell-free extracts of *M. tuberculosis* H37Rv grown *in vivo* are completely devoid of dehydrogenase activity. This was shown to be due to an inhibitory substance confined only to tubercle bacilli grown *in vivo*, and as yet unidentified. Similar inhibitory activity was also found in extracts of mouse lungs infected with *M. tuberculosis*. The inhibitor affected lactic dehydrogenase of cell-free extracts of *M. phlei*, *M. tuberculosis* BCG and H37Rv, and succinoxidase of lung homogenates of normal mice.

Sumario

Estudios del Mycobacterium Tuberculosis H37Rv Cultivado in Vivo

Los extractos citoprivos del *M. tuberculosis* H37Rv cultivado *in vivo* se hallan absolutamente desprovistos de actividad dehidrogenásica. Según se demostró, esto se debía a una substancia inhibidora limitada exclusivamente a los bacilos tuberculosos cultivados *in vivo*, y todavía sin identificar. También se descubrió una actividad inhibidora semejante en extractos de pulmones de ratones infectados por el *M. tuberculosis*. El factor inhibidor afectó la dehidrogenasa láctica de los extractos citoprivos de *M. phlei*, *M. tuberculosis* BCG y H37Rv, y la succinoxidasa de los homogeneatos pulmonares de ratones normales.

Resume

Etudes sur le Mycobacterium Tuberculosis H37Rv poussé in vivo

Des extraits sans cellules de *M. tuberculosis* H37Rv poussé *in vivo* sont complètement dépourvus d'activité deshydrogénante. On a montré que

ceci était du à une substance inhibitrice que l'on trouve seulement chez les bacilles tuberculeux qui poussent *in vivo*, et qui n'est pas encore identifiée. Une semblable activité inhibitrice fut aussi trouvée dans des extraits de poumon de souris infectées par *M. tuberculosis*. L'inhibiteur agit sur la déshydrogénase lactique des extraits sans cellules de *M. phlei*, *M. tuberculosis* BCG, et H37Rv, et sur la succinoxidase des solutions homogéinisées de poumon de souris normales.

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Case Reports

CAVERNOSTOMY[1,2]

A Salvage Procedure for Giant Tuberculous Cavities

J. N. BRIGGS, N. C. HAMEL, AND R. R. TORREY

(Received for publication May 23, 1960)

Introduction

In the present-day enthusiasm for pulmonary resection, cavernostomy has become a rarely utilized surgical procedure. During the past eleven years, 1948 to 1959, only sixteen cavernostomies have been performed at the Olive View Hospital. During a similar period, six hundred and forty-six pulmonary resections were done for cavitary tuberculosis. In spite of the rarity of this procedure, the writers believe that cavernostomy continues to be indicated in certain specialized situations. Cavernostomy in this paper does not refer to the Monaldi procedure, but rather to open cavity drainage performed in one or two stages, turning a skin flap into the cavity. The technique is very similar to that described by Barkley (1).

Briefly, the procedure is indicated in salvage cases when pulmonary resection and thoracoplasty are not suitable. It is useful in far advanced cavitary disease in patients who have poor pulmonary function. Drug resistance and the protection of the uninvolved lung tissue from bronchogenic spreads are further indications for cavernostomy. In the present series, 13 of 16 patients had drug-resistant infections.

Because this series deals with salvage cases only, in most of which the infections were drug resistant, a high morbidity resulted. Sixteen, or 100 per cent, of all of the patients had fever post cavernostomy, varying from two to thirty days. One patient had a postoperative pneumothorax, and another developed hemorrhage from the cavernostomy site. There was no operative mortality. However, 3 late deaths did occur in this series. The deaths resulted from congestive circulatory failure ten months after cavernostomy, progressive tuberculosis twenty-two months after cavernostomy, and bronchogenic carcinoma eleven months after cavernostomy.

Considering that all were salvage cases, the results are rather encouraging (table 1). Four patients have been discharged as well. Two of these patients had cavernostomy alone and 2 had cavernostomy plus other surgical procedures. Three more patients were discharged as improved, 2 with draining cavernostomies and sputum negative for tubercle bacilli, and one with infectious sputum. Five patients are hospitalized but improved and their sputum is noninfectious, and only one patient is hospitalized and unimproved, with infectious sputum.

The following case reports illustrate the type of disease with which this report deals, as well as the results obtained from cavernostomy, both good and bad.

Case Reports

Case 1. A. R. was a young Mexican male who spent eight years of his adult life, from twenty-three to thirty-one years, in the Olive View Hospital. He was admitted to the hospital on January 17, 1945, with far advanced, active tuberculosis. Shortly after admission, a permanent left phrenic crush was done and a pneumoperitoneum was instituted. The cavity on the left promptly healed, but the patient developed cavitary disease in the right upper lobe. Two stages of thoracoplasty (five ribs) were performed on December 14, 1949, and on January 10, 1950, for the right upper lobe cavity, which closed. The patient then developed a lower lobe cavity, which was shown on an October, 1950 film. On November 8, 1950, a cavernostomy was done on the right lower lobe cavity. The sputum, which had been persistently positive for tubercle bacilli and drug-resistant, became noninfectious. It became infectious again, and the cavernostomy was revised on December 7, 1951. Once again the sputum became noninfectious, but only for two months. A second revision of the cavernostomy was completed on July 16, 1952. The patient was discharged from the hospital on May 23, 1953. At the time of discharge, his cavity was completely epithelized and there was no drainage.

[1] From the Olive View Hospital, Olive View, California.

[2] Presented before the Medical Session, as part of *Section 5A*, at the annual meeting of the American Trudeau (now Thoracic) Society, Los Angeles, California, May 17, 1960.

TABLE 1
RESULTS

Discharged well	
Cavernostomy alone	2
Cavernostomy plus other surgery	2
Discharged improved	
Sputum negative for tubercle bacilli	2
Sputum positive for tubercle bacilli	1
Hospitalized improved	
Sputum negative for tubercle bacilli	5
Hospitalized unimproved	
Sputum positive for tubercle bacilli	1

The patient's sputum has remained noninfectious. This illustrates the value of cavernostomy as a curative procedure in an individual who was denied surgical resection because of extensive bilateral disease, a permanent left phrenic crush, and a right thoracoplasty.

Case 2. P. N. was a 22-year-old Negro male who was admitted to the hospital with far advanced cavitary tuberculosis and drug-susceptible tubercle bacilli in the sputum on July 29, 1957. On March 12, 1958, a left anterior cavernostomy was performed for the giant cavity on the left. Cavernostomy was followed on April 24, 1958 with a one-stage, five-rib thoracoplasty on the left. The smaller cavity remained on the right but the sputum became noninfectious. A right upper lobectomy was done on August 6, 1958.

The patient was discharged on February 25, 1959, and has remained well since that date. This case demonstrates the usefulness of cavernostomy when combined with other surgery.

Case 3. L. S. was a 42-year-old white male who entered the hospital August 4, 1955, with far advanced, active, cavitary tuberculosis. His sputum contained drug-resistant tubercle bacilli. On September 16, 1956, a right extraperiosteal Lucite plombage was done. On March 29, 1957, a left anterior cavernostomy was done. Within two months of cavernostomy, the sputum became noninfectious. At the time of discharge from the hospital on August 14, 1958, the cavernostomy sinus continued to drain, but cultures of drainage were repeatedly negative.

Although this patient has the disadvantage of a draining sinus, his sputum is noninfectious and he was sufficiently improved to warrant discharge from the hospital.

Case 4. J. M. was a 55-year-old Mexican housewife who was admitted to Olive View Hospital on November 5, 1956. She had previously (in 1949) had a right thoracoplasty and right upper lobectomy. Her sputum was positive for tubercle bacilli on microscopy and culture. A large left upper lobe cavity was filled with debris. A left anterior cavernostomy was performed in two stages on April 19, 1957, and April 26, 1957. High concentrations of tubercle bacilli were seen in the debris removed from the cavity.

Since operation the cavernostomy sinus has continued to drain, and the patient has had several episodes of moderate bleeding from the sinus. This has necessitated intermittent hospitalization. She has gained weight from 90 to 150 pounds, her sputum has become noninfectious, and the size of the cavity has been reduced.

Case 5. M. C. was a 35-year-old white female who entered the hospital on September 16, 1952. She had been hospitalized continuously for almost eight years. She had far advanced, active, bilateral pulmonary tuberculosis with drug-resistant tubercle bacilli. The left anterior cavernostomy was performed in three stages on July 9, 1957, July 31, 1957, and September 11, 1957. Her sputum became temporarily noninfectious and she gained weight from 97 to 120 pounds.

The patient promptly lost the weight she had gained and her sputum once again became infectious. She also developed tuberculous otitis media. She remains hospitalized and unimproved—a cavernostomy failure.

SUMMARY

The highlights of the experience of the writers with 16 cavernostomies performed in the past eleven years have been briefly reviewed and presented. Because of the size of the series, no statistical conclusions have been drawn. In general, cavernostomy is considered to be a useful procedure in giant cavitary disease when resection is contraindicated because of drug resistance, contralateral disease, and poor pulmonary function. The procedure is effective in inducing reversal of infectiousness and protecting uninvolved lung from bronchogenic spread from a poorly drained cavity. In endorsement of pulmonary resection and collapse procedures, cavernostomy should not be overlooked.

SUMARIO

La Cavernostomía. Procedimiento de Salvamento para las Cavernas Tuberculosas Gigantes

Repásanse y preséntanse aquí sucintamente los puntos culminantes de lo observado por los AA. en 16 cavernostomías ejecutadas en los últimos once

años. Debido al tamaño de la serie, no se sacan conclusiones estadísticas. En general, se considera la cavernostomía como procedimiento útil en la enfermedad de cavernas gigantes cuando está contraindicada la resección debido a fármacorresistencia, afección contralateral y disfunción pulmonar. El procedimiento resulta eficaz para inducir viraje de la infecciosidad y proteger el pulmón indemne contra la difusión broncógena procedente de una caverna mal drenada. En el apoyo de los procedimientos de resección y colapso pulmonares, no debe desatenderse esta operación meritoria.

Resume

Cavernotomie. Un procédé salvateur pour les cavernes tuberculeuses géantes

Les points les plus importants de l'expérience des auteurs sur 16 cavernotomies pratiquées pendant ces 11 dernières années, ont été passés brièvement en revue et présentés. En raison de l'importance de ces séries, aucune conclusion statistique n'en a été tirée. La cavernotomie est considérée en général comme un procédé utile dans la forme de la maladie avec caverne géante, quand la résection en est contrindiquée pour cause de résistance au médicament, de maladie controlatérale, et d'une mauvaise fonction respiratoire. Le procédé agit en incitant le "renversement de contagiosité" et en protégeant le poumon non atteint d'une propagation bronchogène à partir d'une caverne mal drainée. A l'appui de la résection pulmonaire et des procédés de collapsus, cette opération valable ne devrait pas être ignorée.

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CLAVICULECTOMY IN THE MANAGEMENT OF POST-RESECTIONAL SPACE PROBLEMS[1]

JESSE P. CHAPMAN, JR.

(Received for publication June 20, 1960)

Introduction

Certain problems connected with the surgical care of the tuberculous patient present themselves so rarely that there is little opportunity to establish a routine method of treatment for them. Most bronchopleural fistulas respond to standard collapse procedures by closure of the space and control of the sputum or drainage. Occasionally, however, a spatial defect in the uppermost levels of the thorax may be extremely difficult to close because the clavicle acts as a tent-pole to hold the shoulder girdle and its attached soft tissues away from the chest wall (1).

In this sanatorium, this problem was solved on two occasions by claviculectomy, with gratifying results. There seem to be few references to this procedure in the literature, and the present cases are being reported as a means of calling attention to a little-known, little-needed—but extremely useful—adjunct to conventional methods of treatment.

Case Reports

Case 1 (MLV., WNCS #3086). A 24-year-old Negro female first became aware of her tuberculosis in 1948 at the age of fourteen. When she was first admitted to the Western North Carolina Sanatorium in 1951, she had been receiving bilateral pneumothoraces at another institution for several months. The medical staff at the sanatorium elected to discontinue pneumothorax in favor of pneumoperitoneum plus isoniazid and PAS therapy.

Shortly after her admission, the patient left the sanatorium (against medical advice), only to be readmitted in 1955 with a further extension of her disease.

On August 23, 1955, the apical-posterior segment of the left upper lobe, containing a cavity, was removed. Postoperatively, the patient developed empyema and a bronchopleural fistula. A left thoracoplasty was planned for the immediate future, but an exacerbation of her disease was found in the opposite lung in roentgenographic studies done the day before the projected surgery.

In October, 1956, the patient again left the sanatorium without medical consent; but she returned less than a week later. On March 1, 1957, a tailored thoracoplasty was performed on the left side. This procedure failed to help her condition, and she continued along her progressively down-hill course by developing a cavity in the mid-portion of the right lung. The thoracoplasty wound continued to drain large amounts of purulent material. Injections of radiopaque oil into the fistulous tract disclosed a large space which extended behind the clavicle up into the lower part of the neck (figure 1). Wide excision of the fistulous tract with subsequent packing with iodoform and other substances combined with irrigation of the opening with innumerable medications met with failure on two separate attempts, and she was given all known combinations of chemotherapeutic agents to little avail. The cavity in the right lung improved slowly. The fistula on the left did not improve at all.

On June 16, 1959, a left partial claviculectomy was performed.

Following this procedure, the drainage has ceased and the space has closed. For the first time in four years, the left chest is stable (figures 2 and 3). Efforts can now be concentrated on improving the disease in the right cavity.

Case 2 (MC., WNCS #4559). A 53-year-old Negro female was first admitted to the Western North Carolina Sanatorium on May 18, 1955. Two years prior to this admission, she had undergone a right upper and middle lobectomy at another institution (December 17, 1953), and, following that operation, she had developed a right empyema necessitans which perforated spontaneously in the right sub-mammary region and was later drained surgically posterolaterally.

When the patient was first seen at the sanatorium in May, 1955, there were three large draining sinuses in the right upper chest and axilla; and both sputum and sinus drainage were positive for tubercle bacilli on microscopy and culture. Her roentgenograms obtained on admission to this sanatorium (figure 4) showed a space in the right upper chest which was interpreted as representing a bronchopleurocutaneous fistula.

Because of her unfortunate experiences with her previous surgery, she refused to consider any other operative procedures at this time; but in

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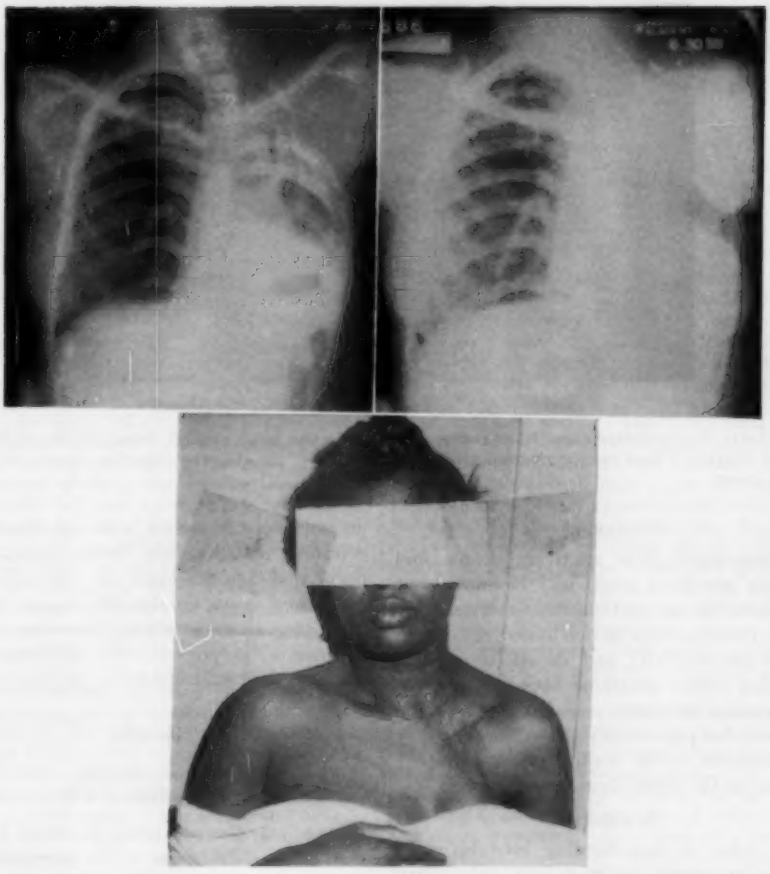


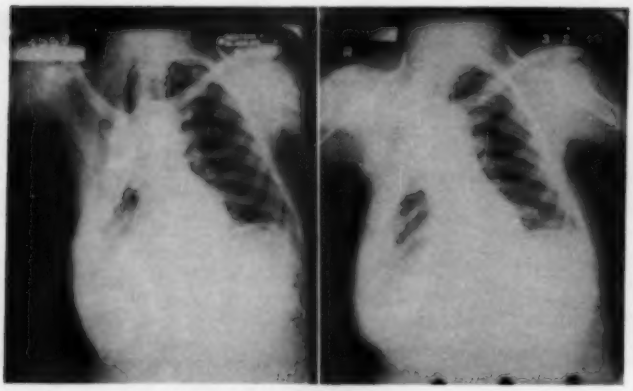
Case 1

FIG. 1 (Upper left). Injection of the sinus tract and space with radiopaque oil prior to claviculectomy.
FIG. 2 (Upper right). Chest roentgenogram two weeks after claviculectomy. There is no space visible on the left.
FIG 3 (Lower). Appearance of left shoulder after claviculectomy.

September, 1955, she consented to a seven-rib thoracoplasty, which she tolerated rather well. Her sinus tracts continued to drain, but her weakened and debilitated status precluded any further surgery until July, 1957, when her thoracoplasty was revised and the sinus tracts were excised and curetted. Despite intensive chemotherapy and daily irrigations of the sinus tracts with chloroazodin (Azochloramid®), she continued to drain from the three openings. Although her sputum was usually negative for tubercle bacilli, a positive sputum culture was obtained in November, 1957.

Believing that closure of the subscapular space was necessary for closure of her draining sinuses and that such closure was being prohibited by the action of the clavicle, a right claviculectomy was performed on October 22, 1958 and the sinus tracts were excised again at the same time. Within six weeks, drainage was insignificant and her subsequent sputum examinations were negative.

By February, 1959, all of the sinus tracts were completely healed (figure 5); and the patient left the sanatorium on April 19, 1959.



Case 2

FIG. 4 (Left). Preoperative chest roentgenogram showing the large spatial defect in the right chest.
FIG. 5 (Right). Chest roentgenogram after claviculectomy, showing the effective closure of the pre-existing space.

DISCUSSION

Claviculectomy is a relatively simple and innocuous procedure which may be carried out under local or general anesthesia with little trauma, even to a sick or debilitated patient. In spite of its simplicity and its effectiveness in controlling certain situations, little has appeared in the medical literature concerning its use. The purpose of this paper is to point out its simplicity and usefulness in the control of certain space problems of the upper thorax.

SUMMARY

Two cases of long-standing retroclavicular, subscapular spaces resulting from tuberculous empyema and postoperative bronchopleural fistula were successfully controlled following resection of the clavicle and further excision of the fistulous tracts. Complete healing occurred in both cases. Little or no shoulder dysfunction appeared to result from this procedure.

SUMARIO

La Claviculectomia en la Asistencia de los Problemas Planteados por los Espacios Post-Reseccionales

Dos casos de espacios subescapulares, retroclaviculares de larga duración, provenientes de un empiema tuberculoso y de una fistula broncopleural postoperatoria fueron atendidos con buen resultado mediante la resección de la clavícula y nueva excisión del trayecto fistuloso. En ambos casos se obtuvo cicatrización total. Al parecer, el procedimiento no ocasionó más que poca o ninguna disfunción del hombro.

RESUME

Claviculectomie dans la conduite à tenir avec des problèmes d'espace consécutifs à une résection

Deux cas d'espace sous-scapulaires et rétro-claviculaires, de longue durée, provenant d'empyème tuberculeux et de fistules bronchopleurales postopératoires furent contrôlés avec succés après la résection de la clavicule et l'excision ultérieure des trajets fistuleux. Une guérison complète se produisit dans les deux cas. Il n'apparut pas ou peu de trouble de la fonction de l'épaule comme résultat de ce procédé.

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CHEST CONFERENCE

University of California School of Medicine

CASE UCH 306071

DR. JOHN RUMSFELD[1]: A 57-year-old man was admitted to the hospital on May 20, 1960, because of numbness in the right axilla of three months' duration and aching pain under the right scapula of six weeks' duration.

History: Three months previously the patient first noted persistent numbness in the right axilla and down the posterior aspect of the upper arm. His physician discovered that he usually worked with his right arm hanging over the back of his chair, and instructed him to avoid this position; thereafter, the numbness slowly disappeared, but the posterior aspect of the upper arm remained slightly tender. Six weeks before entry the patient developed aching pain under the right scapula which became worse in the evening, was partly relieved when he lay on his right side, was aggravated when he lay on his back, and often required codeine for relief. An orthopedic consultant found no skeletal source for the pain. The patient was referred for diagnostic study because of gross abnormalities on an X-ray film of the chest.

Following an injury at the age of ten years, the patient underwent right hip fusion. He consumed 10 ounces of whiskey and smoked 2 to 4 packages of cigarettes per day for many years. He had a post-nasal drip and a cough productive of 2 tablespoons of clear mucoid sputum per day for thirty years. From 1948 to 1955 he had lived in Iowa and Nebraska, and from 1955 to 1960 in the San Joaquin Valley in California.

Physical examination: The patient was a well-nourished male in no distress. His temperature was 37°C., pulse 80, respirations 16, and blood pressure 135 systolic, 80 diastolic in mm. of mercury. The lateral chest expansion was 2 inches, the diaphragmatic excursion 1 inch bilaterally, and the thorax was resonant to percussion. In addition to diffuse wheezing, scattered rhonchi were noted, particularly over the right upper chest. At the medial border of the spine of the right scapula there was a tender area 3 cm. in diameter. The right hip was solidly fused and the underdeveloped right leg was 4.5 inches shorter than the left, producing a moderate lumbar lordosis and scoliosis.

Laboratory findings: The leukocyte count was 8,000, the hematocrit 43 per cent, and the sedimentation rate 14 mm. per hour (Wintrobe). Sputum specimens were obtained for fungal and mycobacterial cultures and for cytologic studies. Coccidioidin, 1:100, and intermediate-strength PPD tuberculin skin tests were negative, but the histoplasmin skin test was positive. Complement-fixation tests for blastomycosis, coccidioidomycosis, and histoplasmosis were negative. The patient was scheduled for biopsy at this time.

DIFFERENTIAL DIAGNOSIS

DR. H. CORWIN HINSHAW, SR.[2]: May we see the X-ray films at this point? Although it is deplored by many, I hold to the opinion that examination of the X-ray films of the chest should be done even before the physical examination if they are available. Indeed, they are a part of physical examination.

DR. CHARLES MEIER[3]: The X-ray films of the chest (figure 1*A*, *B*) show no bony lesions and a normal cardiac silhouette, but there are marked abnormalities in both lung fields. On the right there is a large calcified paratracheal lymph node, widespread fibrosis throughout the lung, and linear scarring in the minor fissure. There is pleural thickening in the upper lobe and an irregular area of consolidation in the posterior apical segment, with linear strands extending down toward the enlarged right hilum. In the left lower lobe there is a thick-walled cavity 3 cm. in diameter with an irregular margin and a possible fluid level.

The roentgenographic findings in the right lung suggest inflammatory disease which is probably old; these could be due to tuberculosis or several fungi, especially coccidioidomycosis or histoplasmosis. The cavitary lesion in the left lower lobe might also be caused by an inflammatory process or a separate entity such as a cavitary neoplasm.

DR. HINSHAW: We have the problem of a middle-aged man—quite an intemperate fellow, even by San Francisco standards—who is eligible for a variety of pulmonary diseases. This very intemperance should affect our judgment. Aspirational lung abscess is almost an occupational hazard of the alcoholic, especially if this is his full-time job. But this record does not supply us with any history of a pneumonia-like illness; there is no mention of fever or voluminous purulent bronchorrhea. An aspirational pyogenic lung abscess is not likely to be so silent, and I am going to discard this possibility on that basis.

This man does not have the roentgenographic sign of recurrent acute alcoholism. This sign is fairly reliable and consists of multiple rib fractures, lateral in location and usually with no history of appropriate trauma.

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[1] Fellow in Chest Diseases of the California Tuberculosis and Health Association and the San Francisco Tuberculosis Association.

[2] Clinical Professor of Medicine, University of California School of Medicine.

[3] Assistant Professor of Radiology, University of California School of Medicine.

His residence in the San Joaquin Valley causes us to suspect that the cavity in the left lung is coccidioidal. Although the cavity is described as "thick-walled," this possibility is not excluded. Thickness is a subjective term, but we see carcinomatous cavities which are quite thin-walled, and coccidioidal or tuberculous cavities which are both thick- and thin-walled. The distinction must not be made on such grounds alone. The lack of a positive coccidoidin skin test is cause for slight hesitation, yet we are all seeing more coccidioidal cavities with negative skin tests than we thought possible fifteen years ago (1). The lack of positive coccidioidal serology does not concern us at all, for up to 40 per cent of cases of cavitary coccidioidomycosis will yield negative serologic tests, at least late in the disease (1, 2). We may not know whether this cavity is coccidioidal unless it is resected, but I feel sure that it will not be resected when he has so much serious disease on the other side.

His histoplasmin skin test is positive. Is it possible that this is caused by a cross reaction with coccidioidal infection? I think not, because when cross reactions occur, both tests are positive. Does this man have cavitary histoplasmosis? I cannot exclude this possibility, even with the negative serology. However, a sustained high titer of complement-fixing antibodies is the rule in reinfection histoplasmosis (3). Lacking this finding, I am discarding this as a probability. Only if *Histoplasma capsulatum* were found in the sputum would I revive the idea. The calcified paratracheal node is, I believe, merely a fossil of the patient's healed histoplasmosis.

Tuberculosis is a very common cause of cavitary lung disease, and, typically, cavities appear in the superior segment of the lower lobe. Tuberculosis is about the only disease which could explain all of this man's pulmonary symptoms, as they are described in the roentgenologist's report. This describes a dense scarring and contracting upper lobe lesion in the apex and a cavity in the left lower lobe. But the tuberculin skin test was negative. Such negative tests should always be repeated because of the possibility of technical errors. The next edition of *Diagnostic Standards and Classification of Tuberculosis* will not recommend a more concentrated test to follow a negative intermediate PPD skin test, but will recommend a repetition of the intermediate PPD test first. (When this is done, you may be surprised at the number of false negative tests.) In this case we must accept the statements in the record as gospel truth. Therefore, I cannot accept tuberculosis as a probability. If this were a tuberculous cavity, it should yield a positive sputum and the skin test should be positive. Perhaps acid-fast bacilli were found. If so, I doubt that they were *Mycobacterium tuberculosis*. Unclassified acid-fast bacilli can produce disease like this and I suspect that they are the cause of some of the rarely reported cases of tuberculin-negative "tuberculosis."

The history of hip fusion is disconcerting because I suspect that it was done for the clinical diagnosis of tuberculosis, and I wonder if the fusion would have been done if a tuberculin skin test had been applied by the orthopedist. He surely would have done the test today, but this is forty-seven years later.

We have postponed consideration of this patient's principal complaint and the right apical lesion. I believe that these are connected and that his pain is due to that apical lesion. Irritation of the lower trunk of the brachial plexus would produce his neurologic symptoms. This trunk lies close to the apex of the pleural cavity medially.

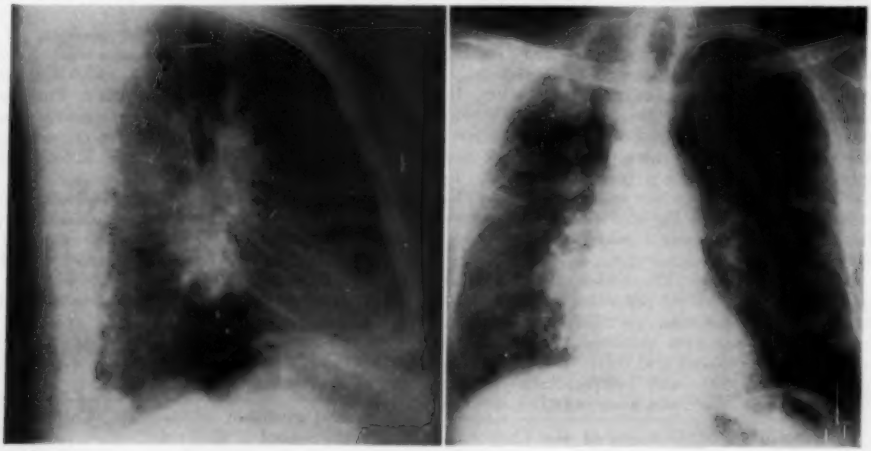

FIG. 1. Roentgenograms of the chest taken May 21, 1960.

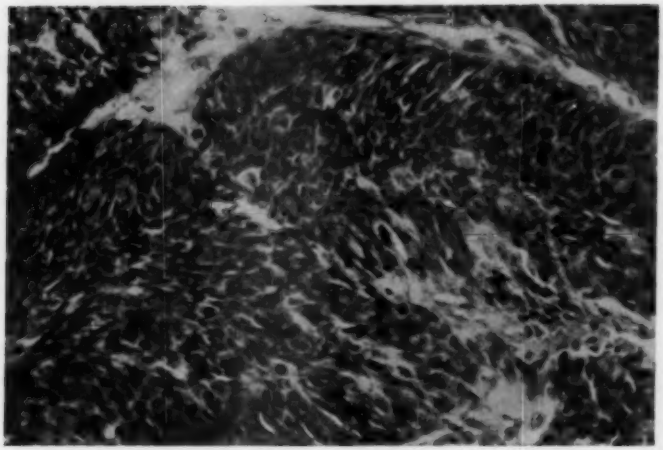

FIG. 2. Section through biopsy specimen. (× 330)

It is made up of the eighth cervical and the first thoracic spinal nerves. Axillary and scapular pain and numbness are characteristic of apical lung disease affecting the brachial plexus. Later, when the sympathetic chain becomes affected, Horner's syndrome appears and erosion of adjacent ribs can often be demonstrated; when the motor nerves to the hand are affected, we have the full-blown picture of Pancoast's syndrome (4). In common practice we have come to refer to this as Pancoast's syndrome when pain is the only clinical symptom. Minor brachial plexus pains may be caused by a variety of conditions. Only cancer is likely to produce severe pain, increasing pain, and pain which requires narcotics. It is difficult to quantitate pain when talking to a patient and more difficult to evaluate the intensity of pain from reading a protocol, even a concise, clearly written report like this one. If this man told me that his pain was like a severe toothache, I would be convinced that he had a cancer. Pain which is worse at night, pain which is intensified when lying down, and pain which requires codeine sounds like the pain of malignancy. Alcoholic neuritis and "Saturday night palsy" do not seem likely, despite the posture described when the man was at work.

I believe that this man had a carcinoma at the apex of the right lung. Most such carcinomas are of peripheral bronchogenic origin, primary at this site. I have seen Pancoast's syndrome caused by metastatic carcinoma, either from the lung or, in one case at least, from the breast. It is possible that this man had a primary carcinoma in the left lower lobe with metastasis to the right apical area. Indeed, the principal lymphatic drainage channels from the left lower lobe lead to the base of the neck on the right side, rather than the left, in many persons (5).

My choice of diagnoses is now narrowed to carcinoma of the left lung with metastasis to the right apex or to carcinoma primary at the right apex with another disease in the left lung. That other disease would most likely be coccidioidomycosis. We cannot exclude a host of air-containing lesions, including lung cysts, indolent abscesses, and other conditions which would be impossible to suspect on the basis of available information.

My final opinion, based on frequencies, is the commonest cause of apical lung densities with brachial plexus pains—bronchogenic carcinoma. The second diagnosis is the commonest cause of symptomless lung cavities among residents of the San Joaquin Valley—residual cavitary coccidioidomycosis.

Dr. Hinshaw's diagnosis: Bronchogenic carcinoma, right upper lobe, apical and posterior segments; coccidioidomycosis, with residual left lower lobe cavity.

Anatomic diagnosis: Bronchogenic carcinoma, anaplastic type, with local extension to the chest wall.

DISCUSSION

DR. JOHN RUMSFELD: Because of the persistent pain over the distribution of the right third thoracic segment, X-rays for rib detail were obtained which demonstrated a pathologic fracture of the third rib located behind, and largely obscured by, the right apical lesion. On review of the original X-ray films, this fracture was barely visible. The sputum cultures for fungi and acid-fast bacilli were subsequently reported negative.

DR. ROBERT WRIGHT[4]: On cytologic exami-

[4] Assistant Professor of Pathology, University of California School of Medicine.

nation, the first sputum specimen contained a number of small hyperchromatic cells with scanty cytoplasm and was reported as suspicious for malignant cells. During biopsy of the rib at the fracture site, gross neoplastic infiltration of the rib and chest wall was evident. Examination of the biopsy specimen revealed an anaplastic small-cell (oat-cell) carcinoma (figure 2). Three sputum specimens were subsequently reported positive for malignant cells.

DR. HINSHAW: Did this man ever develop Horner's syndrome?

DR. RUMSFELD: He did not.

DR. CABOT BROWN: Dr. Hinshaw, you said that this could be either a primary carcinoma in the left lower lobe with metastases to the right upper lobe, or a primary carcinoma in the right upper lobe with a different disease in the left lower lobe. Why can't metastases occur equally well from the right upper to the left lower lobe? Why isn't the whole picture explained by carcinoma?

DR. HINSHAW: My reasoning was based on two observations. The first is that, while cavitation in primary pulmonary carcinoma is common (6), cavitation in metastatic pulmonary carcinoma is rare, although it has been reported (6, 7). The second is that the logical route of lymphatic spread is from the left lower lobe to the right apical area, rather than the reverse.

DR. CHARLES T. CARMAN: In the two weeks following the biopsy, the patient developed complete paraplegia of the lower extremities with a sensory level at T_8. He died at home two months later. Unfortunately, no autopsy was obtained; however, Dr. Meier has X-ray films of the chest taken seven weeks after admission which show some interesting changes.

DR. MEIER: Since the previous study, both hilar shadows increased in size and a new infiltrate appeared in the left upper lobe. A small pleural effusion was present on the right. X-ray films of the spine showed no bony lesions.

DR. HINSHAW: Spinal cord symptoms are fairly common manifestations of metastatic bronchogenic carcinoma, usually without evidence of involvement of bony structures. I think most of these are true metastases to the spinal cord

In view of the additional information just given, I believe we can reconstruct the sequence of events in this patient as follows: Initially he probably had a cavitating primary carcinoma in the left lower lobe. Metastases to the right hilum and right apex occurred, and finally extension of the apical lesion to the chest wall produced the presenting symptoms of numbness and pain. Unfortunately, as is so often the case in cancer of the lung, the disease at this time was already far advanced.

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September 30, 1960

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Notes

STUDIES IN SULFUR METABOLISM OF MYCOBACTERIUM TUBERCULOSIS, H37RV STRAIN, AND DEVELOPMENT OF A SYNTHETIC MEDIUM DESIGNATED AS "M-MEDIUM"

Various liquid media, such as the Youmans, Sauton, Proskauer-Beck, Kirchner, and Dubos media, have been used for the growth and maintenance of *Mycobacterium tuberculosis*. The British Medical Research Council has used a modified Dubos medium (hereinafter called MRC-Dubos medium) extensively for the growth and *in vitro* screening of possible antituberculous compounds. The presence of casein hydrolysate fails to give this medium a truly synthetic character suitable for metabolic studies. Enzymatic casein hydrolysate, besides containing approximately nineteen amino acids in varying proportion, is also likely to contain other intermediate complex organic substances along with inorganic salts, depending on whether the casein is rennet- or lactic acid–derived. Casein is an animal protein with the approximate percentage composition[1]—N—15.4 per cent and S—0.75 per cent, the source of sulfur being principally methionine, which constitutes 3.5 per cent of the total amino acids.

The effects of various compounds, viz., carbohydrates, hydrocarbons, organic acids,[2-9] et cetera, on the growth, oxygen uptake, and metabolism of *M. tuberculosis* were studied in detail. Investigations of the role of various organic and inorganic nitrogen compounds[7, 10-13] on the microbe indicated that certain amino acids, particularly glutamic and aspartic acids, can serve as the sole source of nitrogen.

Liquid media used for growth of *M. tuberculosis* usually contain significant quantities of sulfur either in the form of magnesium or potassium sulfate. Although it has been found that the presence of magnesium[8] favorably influences the growth of *M. tuberculosis*, very little attention until now has been paid to the true role of sulfur in such combinations. The amino acid composition[14-19] of *M. tuberculosis* and other microorganisms grown in a variety of media has been examined, and sulfur-containing compounds, such as methionine, cystine, and glutathione, were obtained both in culture filtrate and cell-hydrolysate homogenates—a fact which lends further support to the probable usefulness of sulfur in the medium.

The present work describes in detail the role of sulfur, in inorganic and organic combinations, on the growth of *M. tuberculosis* strain H37Rv. The role of sulfur as magnesium sulfate was studied in synthetic Medium II of the following composition:

KH_2PO_4–1.0 gm.; Na_2HPO_4, $12H_2O$–6.25 gm.; sodium citrate–1.5 gm.; $MgSO_4 \cdot 7H_2O$–0.6 gm.; Tween 80® (10 per cent solution)–5 ml.; L (−) asparagine–0.2 gm.; DL-alanine–0.15 gm.; DL-tryptophane–0.030 gm.; glycine–0.02 gm.; l (+) glutamic acid–0.06 gm.; glass-distilled water–1,000 ml. pH 7.3–7.4.

Withdrawal of magnesium sulfate or its substitution by magnesium citrate in Medium II did not result in any growth of strain H37Rv, lending further support to the essential role of sulfur in this medium with respect to strain H37Rv. It was noticed, however, that the absence of magnesium

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sulfate in MRC-Dubos medium containing casein hydrolysate did not alter the rate of growth of *M. tuberculosis* strain H37Rv. This suggested that sulfur-containing amino acids, as present in casein hydrolysate solution, are perhaps helping the growth as a source of sulfur in the absence of magnesium sulfate. Accordingly, a new, purely synthetic medium designated as "M-Medium" was developed, substituting magnesium sulfate with a suitable concentration of DL-methionine (0.38 gm. per liter) or DL-acetyl methionine (0.48 gm. per liter), with the inclusion of five amino acids as indicated in Medium II. The methionine concentration of "M-Medium" has been calculated on the equivalent sulfur content of magnesium sulfate as present in Medium II.

Mycobacterium tuberculosis strain H37Rv was maintained for more than two years in "M-Medium" through subculture after every fourteen days in the medium. No change either in the morphologic character or in the staining properties of the Mycobacterium was observed as compared to tubercle bacilli similarly maintained in MRC-Dubos medium.

Strain: Mycobacterium tuberculosis strain H37Rv maintained in MRC-Dubos medium was used.

Inoculation: Five milliliters of a fourteen-day-old fresh culture of H37Rv in MRC-Dubos medium were diluted with 5 ml. of sterile MRC-Dubos medium, and 0.1 ml. of this diluted suspension of the Mycobacterium was used as inoculum.

TABLE 1

EFFECTS OF VARIOUS SULFUR COMPOUNDS ON THE GROWTH OF M. TUBERCULOSIS STRAIN H37Rv

Serial Number	Sulfur Compounds Added to Basal Medium	Concentration of Sulfur Compounds in Mg. per 100 Ml. of Medium	Growth Reading
1	Magnesium sulfate, $7H_2O$	60	10
2	DL-Acetyl methionine	48	9–10
3	DL-Methionine	38	9–10
4	D (+) Methionine	38	8
5	l(−) Methionine	38	7
6	l(−) Cystine	30	0–4 Extremely irregular
7	l(+) Cysteine HCl	40	0–4
8	Thioglycollic acid	22.4	7
9	Thiamine hydrochloride	90	0
10	Thiourea	18.5	0
11	Thiosemicarbazide	22	0
12	Sodium sulfate (exsiccated)	38	6
13	Sodium thiosulfate $5H_2O$	30	5
14	Ammonium sulfate	32	7
15	Potassium thiocyanate	24	4–5
16	Ferrous ammonium sulfate	45	5
17	Medical Research Council-modified Dubos medium served as control		10
18	Basal medium		0

TABLE 2

A COMPARATIVE STUDY OF THE IN VITRO SCREENING OF ANTITUBERCULOUS COMPOUNDS WITH "M-MEDIUM"

Serial Number	Test Compounds	Minimal Inhibiting Concentration of the Compound in Mg. per 100 Ml. of the Medium			
		M-Medium		Medium-I	
		With Serum	Without Serum	With Serum	Without Serum
1	Isoniazid	0.0078	0.0078	0.0078	0.0078
2	Sodium PAS dihydrate	0.0312	0.0312–0.0156	0.0312	0.0312–0.0156
3	Thiacetazone	0.0625	0.0625	0.0625	0.0625
4	Cyanacetic acid hydrazide	0.125–0.0625	0.0625	0.125–0.0625	0.0625

Medium: Five milliliters of the respective experimental medium were used in each 25-ml. screw cap vial, autoclaved at 10 pounds' pressure for ten minutes, and incubated for sterility testing for seventy-two hours at 37°C. prior to inoculation.

Growth record: Five milliliters of the respective sterile experimental medium were distributed in each 25-ml. screw cap vial. Six such vials for each set of experiments were used, inoculated, and incubated at 37°C. The growth reading was recorded on visual observation on an average of growth in six vials after fourteen days of incubation, and is expressed in comparison to standard growth in MRC-Dubos medium, taking the latter to be 10. The growth reading, as recorded in tables 1 and 3 as 0, 1, 2, 3, 4, and so on, represents no growth, 10 per cent, 20 per cent, 30 per cent, 40 per cent, and so on, of the growth as found in MRC-Dubos medium. "M-Medium" without DL-acetyl methionine was used as basal medium.

Different organic and inorganic sulfur compounds were added to the basal medium in amounts approximately equivalent to the concentration of magnesium sulfate in terms of its sulfur content present in Medium II, and their effects on the relative growth of strain H37Rv are recorded in table 1. The basal medium without any source of sulfur, however, did not support growth of H37Rv.

* * *

The sulfur compounds studied varied in their capacities to support growth of *M. tuberculosis* strain H37Rv. Magnesium sulfate, DL-acetyl methionine, or DL-methionine had more or less similar capacities to support good growth. Growth with 1(−) cystine and 1(+) cysteine HCl was extremely erratic and irregular. Some of the vials showed no growth, while a maximum of about 40 per cent growth as compared with that in MRC-Dubos medium was found in other vials. The influence of various concentrations of DL-acetyl methionine—between 96 mg. and 10 mg. per 100 ml. of medium—on the growth of strain H37Rv was studied separately, and it was found that increasing the concentration to more than 48 mg. per 100 ml. of the medium had no stimulating effect on growth. Lowering the concentration to less than 30 mg. per 100 ml., however, had a retarding effect on the growth of strain H37Rv.

A comparative study of the *in vitro* screening of antituberculous compounds with "M-Medium" is shown in table 2.

The results in table 2 indicate the suitability of "M-Medium" for *in vitro* studies of antituberculous compounds in the absence or in the presence of serum.

The influence of different combinations of five sulfur-free amino acids, as present in "M-Medium," on the rate of growth of strain H37Rv was studied. The composition of the basal medium used for this experiment was:

KH_2PO_4–1.0 gm.; Na_2HPO_4, $12H_2O$–6.25 gm.; sodium citrate–1.5 gm.; Tween 80 (10 per cent solution)–5.0 ml.; DL-acetyl methionine–0.48 gm.; magnesium citrate $5H_2O$–0.71 gm.; glass-distilled water–1,000 ml. pH 7.3–7.4.

Five amino acids were incorporated in the basal medium either separately or in combination, and their effect on growth has been expressed in table

TABLE 3

EFFECTS OF FIVE AMINO ACIDS ON THE GROWTH OF M. TUBERCULOSIS STRAIN H37Rv

Serial Number	1(−) Asparagine	DL-Alanine	DL-Tryptophane	Glycine	1(+)-Glutamic Acid	Growth Reading
	mg. per 100 ml.	*mg. per 100 ml.*	*mg. per 100 ml.*	*mg. per 100 ml.*	*mg. per 100 ml.*	
1	20	—	—	—	—	4–5
2		15				3
3			3			4
4				2		2
5				10		4
6					6	5
7					15	5
8	20	15				6
9	20		3			6
10	20			2		3
11	20				6	6
12		15	3			4
13		15		2		4
14		15			6	4
15			3	2		3
16			3		6	5
17				2	6	4
18	20	15	3			6–7
19	20	15		2		6–7
20	20	15			6	7–8
21	20	0	3	2		6–7
22	20		3		6	7–8
23	20			2	6	7
24		15	3	2		6
25		15	3		6	6
26		15		2	6	6
27			3	2	6	6
28	20	15	3	2		8–9
29	20	15	3		6	8–9
30	20		3	2	6	8–9
31	20	15		2	6	8–9
32		15	3	2	6	8–9
33	20	15	3	2	6	9–10
34	Basal medium					1–1.5
35	Control in Medical Research Council—Modified Dubos Medium					10

3, as compared with the growth in MRC-Dubos medium as standard. Studies were also carried out to find out whether DL-acetyl methionine or DL-methionine as sole sources of both nitrogen and sulfur atoms in the basal medium can support growth of strain H37Rv. It was found that, between concentration ranges of 70 to 40 mg. per 100 ml. of the medium, both can initiate growth. The rate is the same up to four-five days of incubation as compared with the growth in MRC-Dubos medium (which is about 10–15 per cent of the final fourteen days of growth in MRC-Dubos medium). On further incubation in the methionine medium, the mycobacteria seemed to disintegrate.

In view of the inability of either DL-acetyl or DL-methionine, as sole sources of both nitrogen and sulfur atoms, to support growth of strain H37Rv in the above basal medium, the effects of the addition of other amino acids on growth were studied. It was observed from the results in table 3 that the association of certain amino acids, as present in "M-Medium," with DL-acetyl or DL-methionine, either singly or in combination, significantly helps to improve and promote growth. Combinations of any four of the five sulfur-free amino acids in concentrations present in "M-Medium" with DL-acetyl or DL-methionine supported 80-90 per cent growth, whereas suitable combinations of three, two, and one amino acid supported 70–80 per cent, 60 per cent, and 50 per cent of growth, respectively, as compared with the control standard growth in MRC-Dubos medium.

In summary: A purely synthetic medium with DL-acetyl or DL-methionine as the sole source of sulfur atoms, together with five other amino acids, has been developed and is designated as "M-Medium." This medium has been found suitable for growth, maintenance, and *in vitro* screening of antituberculous compounds with *Mycobacterium tuberculosis* strain H37Rv. The sulfur metabolism of strain H37Rv was studied with various organic and inorganic sources of sulfur in place of methionine in "M-Medium." It was found that this particular organism exhibits certain specificity toward different sulfur compounds for its proper growth. Of the sulfur compounds tested, magnesium sulfate and methionine were found to be best suited. The effects of various combinations of the five sulfur-free amino acids, present in "M-Medium," with DL-acetyl methionine were also studied.

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SYNERGISTIC COMBINATIONS OF PENICILLIN AND ISONIAZID IN VITRO AGAINST M. SMEGMATIS

This study was initiated as an indirect approach to the subject of the existence of a synergistic relation between penicillin and isoniazid and other drugs *in vitro*. The literature shows a lack of agreement regarding the effect of penicillin on *M. tuberculosis*.[1-5] More recently, Vaichulis and Doll[6, 7] found that two of the media for *M. tuberculosis* inactivated penicillin rapidly. They pointed out that all present media contain factors which are known to inactivate penicillin within a few hours to several days.[8-16] Hence, technical difficulties with the present media and methods make the *in vitro* study of the possible existence of a synergism between penicillin and isoniazid difficult. Accordingly, it was decided to use an agar-plate diffusion method[17, 18] with a fast-growing acid-fast Mycobacterium to ascertain whether a synergism might exist and, if it existed, to find a method applicable to the slow-growing *M. tuberculosis*.

Test organism: A strain of *Mycobacterium smegmatis* from the culture collection of the University of Illinois Department of Microbiology was used in this study. It was transferred several times through Dubos broth base with Tween® 80 enriched with Dubos albumin medium before being used for susceptibility studies.

Seeding plates: To 100 ml. of nutrient agar or Dubos oleic–agar medium, 1 ml. of the supernatant liquid from a forty-eight-hour Dubos Tween-albumin culture of *M. smegmatis* was added. Ten-milliliter portions were then pipetted into disposable plastic Petri dishes. The plates were allowed to set thirty minutes at room temperature to solidify before drug-susceptibility discs were placed on the surface.

Discs: Discs[19] (6 mm. in diameter) of highly absorbent, pure filter paper designed for the assay of penicillin[20] were used.

Drug combinations tested: Crystalline potassium penicillin G[21] and isoniazid[22] alone or in combination were tested for antimicrobial activity.

Method of drug-susceptibility determination: All test dilutions were made in phosphate buffer[23] at pH 6, an environment that favors penicillin stability. A fresh vial of penicillin was used for each drug-susceptibility test. The discs were immersed in the test solutions for fifteen seconds while being held with sterile forceps. Excess solution was then shaken off and the disc was placed in position on the seeded plate. Readings were made after twenty-four and forty-eight hours of incubation.

The susceptibility of the organism to the various drug combinations tested was determined by comparing the diameters of the zones of inhibition with those of the controls and the other test combinations. For purposes of analyzing the data obtained from these tests, two distinct types of zones were recognized: a clear zone of inhibition with no resistant organisms growing in it, and a hazy zone of inhibition with resistant organisms growing within the zone.

Drug-susceptibility studies showed that isoniazid concentrations between 40 and 50γ per ml. produced clear zones of inhibition 9 to 11 mm. in diameter which persisted throughout forty-eight hours of incubation. Concentrations of 30γ of isoniazid per ml. usually did not produce clear zones of inhibition after twenty-four hours of incubation, and produced only faint hazy zones 8 to 9 mm. in diameter after forty-eight hours of

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TABLE 1

ENHANCING INFLUENCE OF PENICILLIN ON THE EFFECT OF ISONIAZID ON THE GROWTH OF M. SMEGMATIS

Penicillin		Isoniazid	Type of Medium			
			Dubos Oleic-Agar		Nutrient Agar	
			Diameter of Zone of Inhibition in Mm.			
			24 Hours	48 Hours	24 Hours	48 Hours
γ per ml.	*units per ml.*	*γ per ml*				
3	(5)	+10	0	0	0	0
16	(25)	+10	0	0	0	0
32	(50)	+10	0	0	0	0
64	(100)	+10	0	0	0	0
125	(200)	+10	0	0	−7	−7
3	(5)	+20	8	−7 (Hazy)	+7	+7
16	(25)	+20	8	7 (Hazy)	+7	+7
32	(50)	+20	8	+7	8	8
64	(100)	+20	8	8	8	8
125	(200)	+20	8	8	9	9
627	(1,000)		0	0	0	0
		20	8 (Hazy)	0	8 (Hazy)	0

TABLE 2

ENHANCING INFLUENCE OF PENICILLIN ON THE EFFECT OF ISONIAZID ON THE GROWTH OF M. SMEGMATIS

Penicillin		Isoniazid	Type of Medium			
			Dubos Oleic-Agar		Nutrient Agar	
			Diameter of Zone of Inhibition in Mm.			
			24 Hours	48 Hours	24 Hours	48 Hours
γ per ml.	*units per ml.*	*γ per ml.*				
3	(5)	+20	8 (Hazy)	7 (Hazy)	−7	−7 (Hazy)
32	(50)	+20	8	−7	7	7
64	(100)	+20	8	7	7	7
3	(5)	+30	8	8	−7	−7
32	(50)	+30	9	9	8	8
64	(100)	+30	11	10	9	9
627	(1,000)		0	0	0	0
		20	8	0	0	0
		30	9	8 (Hazy)	7	7 (Hazy)
		50	11	11	12	12

incubation. When isoniazid was used in concentrations of 20γ per ml. and lower, faint hazy zones of inhibition 7 to 8 mm. in diameter appeared at twenty-four hours; by forty-eight hours these zones had disappeared entirely.

Therefore, this strain of *M. smegmatis* was susceptible to a minimum of 30γ of isoniazid per ml. and unsusceptible to 20γ per ml. and less, using this method of testing. It is apparent that this strain of *M. smegmatis* was refractory to isoniazid since it required 30γ of the drug per ml. to inhibit growth. Virulent strains of mycobacteria are susceptible to concentrations of isoniazid as low as 0.05γ per ml.

From the results it is apparent that penicillin alone produced no zone of inhibition against *M. smegmatis* even in a concentration of 1,000 units per ml. Furthermore, 20γ of isoniazid per ml. were not inhibitory alone. But, 20γ of isoniazid per ml. in combination with as little as 50 units of penicillin per ml. produced a clear zone of inhibition 7 to 8 mm. in diameter which persisted throughout forty-eight hours of incubation. It is of interest

that penicillin–isoniazid zones persisted through several weeks of further incubation.

Similar findings are noted for other concentrations of penicillin and isoniazid. It is observed that this effect was not achieved with combinations of penicillin and isoniazid when the isoniazid concentration was below 20γ per ml. and the penicillin concentration as high as 100 units per ml. On nutrient agar, a zone of 7 mm. in diameter usually occurred with 10γ of isoniazid per ml. in combination with 200 units of penicillin per ml. The results of typical susceptibility determinations may be seen in tables 1 and 2.

* * *

Synergism between penicillin and isoniazid is apparent from the results because penicillin shows no antimicrobial activity against *M. smegmatis* by itself even in a concentration of 1,000 units per ml. Concentrations of isoniazid which were subinhibitory showed antimicrobial activity when combined with penicillin. When the concentration of isoniazid remained constant, the zone size increased as the concentration of penicillin increased.

The possibility of a merely additive effect is eliminated because penicillin showed no antimicrobial activity at the concentrations tested, which may be considered very high concentrations. This indicates that penicillin potentiates the action of isoniazid.

Numerous reports[8-16] have revealed that various antagonists which rapidly inactivate penicillin are present in media customarily used for culturing mycobacteria. Since it is a slow-acting antimicrobial,[8, 24] the maintenance of its stability under test conditions is essential.

It is especially noteworthy that in all of the papers cited with regard to the effect of penicillin on the tubercle bacillus *in vitro*[1-5] no quantitative determinations were made to determine the amount of active penicillin remaining after the period of incubation. Thus, it is apparent that these studies are not of much value in lieu of the labile nature of penicillin and the absence of determinations to ascertain the amount of active penicillin available.

The mode of action of this synergism will not be considered for, as several reviewers point out,[25-27] too little is known to do so at present. Although penicillin does not appear to have any antimicrobial activity per se, its potency or that of some derivative must remain high in order to participate in synergism.

An obvious criticism of this work is that *M. smegmatis* has been used and the effects of chemicals on it may not apply to *M. tuberculosis*. But as King and associates[16] point out, as long as this possibility is borne in mind, the advantages of this technique in obtaining preliminary information outweigh the disadvantages. To perform the same experiments with *M. tuberculosis* would require experiments which would not give results for several weeks. However, these observations on *M. smegmatis* will be extended to *M. tuberculosis*. If this indirect approach does not prove to be effective in regard to *M. tuberculosis*, then some way will have to be developed to produce a medium in which penicillin is more stable.

In summary: Penicillin acts synergistically with isoniazid against the growth of *M. smegmatis*. The concentrations of penicillin which participated in the synergism with isoniazid are therapeutically attainable. The effect of the synergism between penicillin and isoniazid was augmented as the penicillin concentration was increased, the isoniazid concentration remaining constant. Penicillin showed no antimicrobial activity against *M. smegmatis*, even in a concentration of 1,000 units per ml., when studied under the conditions used in this investigation.

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AN IMPROVED BREATH-ACTUATED BELLOWS APPARATUS FOR THE DETERMINATION OF FORCED EXPIRATORY CAPACITIES

The bellows vital capacity apparatus described by Wang and Shipley[1] and the modifications as described by Horton and Phillips[2] present serious drawbacks for bedside and office use. The method involves holding an instrument at the patient's mouth and at the same time trying to judge just when to depress the button to start the transverse movement of the chart. If the button is depressed too soon, a large segment of the chart is wasted; if not depressed in time, the timing is not accurate. In the calculation of the records, it is time consuming and annoying to count the boxes to determine the end of the one-, two-, and three-second intervals. At present, it is necessary to count 10 spaces to determine where the end of the first second is located, 20 spaces for the end of the second second, and 30 spaces for the location of the end of the third second. The calculation of the maximal expiratory flow rate involves drawing parallel lines, which is in itself time consuming and subject to error. The modifications described in this report are designed to provide convenient, rapid, and accurate methods of performing the tests and calculating the results.

Modifications

Holding of apparatus: The instrument was made useable from a table top. The Vitalor[3] comes supplied with an oval mouthpiece. This was replaced by a round metal mouthpiece to which was attached a 30-inch length of ¾-inch (internal diameter) smooth rubber tubing. The apparatus can now be placed on a table top and the forced vital capacity, the forced expiratory capacities, and the total vital capacity can be performed with the patient in the supine, sitting, or standing position.

Transverse movement of the chart: The push button was replaced by an off-on switch and a snap-action switch,[4] which was activated by a copper rod attached to one corner of the metal cover of the Vitalor apparatus (figure 1). This arrangement did not change the amount of air that must be introduced before any displacement of the lid will be observed. Wang and Shipley[1] found this to be approximately 150 cc. of air. This is recognized by the manufacturers of the apparatus, who have calibrated their pressure-sensitive paper to start recording at the 200-cc. mark. The off-on switch eliminates the necessity of having to keep the button depressed during the complete time of the expiration when the expiration is completed in less than six seconds, and permits switching off the motor to record the complete volume in patients with markedly prolonged expiration time.

The snap-action switch was mounted on a plastic board connected to the base of the apparatus. The internal wiring of the apparatus was not disturbed. One side of the line cord was interrupted and the snap-action switch was inserted so as to create a normally closed circuit. A thin copper bar (1 cm. wide) was soldered to the micro-switch actuator in a crosswise position. This served as a broad base for the copper rod attached to the lid of the apparatus.

Calculations: A modified ruler, based in part on the Segal-Herschfus ruler[5] was designed (figure 2). This ruler permits more rapid and convenient measurement of the time intervals necessary to measure the volume of the forced expiratory capacities for the one-, two-, or three-second intervals. It also makes it unnecessary to draw parallel lines for the measurement of the maximal expiratory flow rate. With this plastic ruler it is no longer necessary to count spaces. The plastic is ruled as shown in figure 2 so that the first second, the second second, and the third second are automatically in place when the start of the exhalation is lined up with the zero line. In the calculation of the maximal expiratory flow rate, all that is now necessary is to draw one line through the points where the graph crosses the broken line at the 1,200-cc. position and where the graph rises from the base line. This line is drawn from the bottom edge of the paper to the top of the paper. The paper is inserted under the plastic ruler and moved along until the line drawn is running through the circle at the lower left-hand corner of the ruler. The maximal expiratory flow rate can now be read directly from the ruler without drawing parallel lines.

* * *

The described modifications to an excellent instrument for office or bedside measurement of an important parameter of pulmonary function

[1] Wang, R. I. H., and Shipley, R. E.: JAMA, 1958, *167*, 1730.

[2] Horton, G. E., and Phillips, S.: Amer Rev Resp Dis, 1959, *80*, 724.

[3] Vitalor,® McKesson Appliance Company, Toledo, Ohio.

[4] Honeywell Micro Switch, Cat. No. BZ-RW 13, Minneapolis Honeywell Regulator Company, Freeport, Illinois.

[5] Segal, M. S., *et al.*: Dis Chest, 1952, *22*, 123.

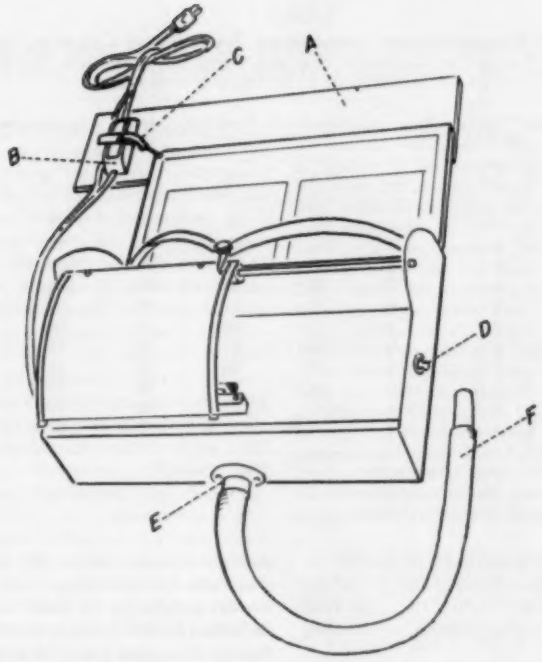

FIG. 1. Modified Vitalor apparatus.
A: Plastic base for mounting snap-action switch.
B: Snap-action switch.
C: Activating arm for snap-action switch.
D: Push-button replaced by off-on switch.
E: Oval mouthpiece replaced by round metal tubing, ¾ inch (outside diameter).
F: Rubber tubing, ¾ inch (internal diameter).

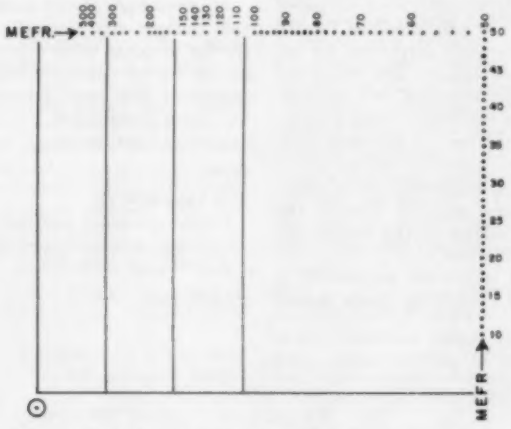

FIG. 2. Plastic ruler to facilitate Vitalor calculations.

TABLE 1

COMPARISON OF TOTAL VITAL CAPACITY, ONE-SECOND TIMED VITAL CAPACITY, AND THREE-SECOND TIMED VITAL CAPACITY USING THE GAENSLER-COLLINS TIMED VITALOMETER, THE MODIFIED VITALOR, AND THE VITALOR WITH OVAL MOUTHPIECE

Patients	Total Vital Capacity Liters			One-Second Timed Vital Capacity Per Cent of Total Vital Capacity			Three-Second Timed Vital Capacity Per Cent of Total Vital Capacity		
	A*	B†	C‡	A	B	C	A	B	C
1	3.1	3.1	3.1	81	81	77	94	94	90
2	3.3	3.3	3.3	64	62	59	82	82	82
3	4.7	4.7	4.7	92	92	86	100	100	99
4	4.7	4.7	4.7	83	83	79	100	99	99
5	2.6	2.6	2.6	73	71	67	92	92	90
6	3.5	3.5	3.4	70	69	67	88	88	87
7	4.0	4.0	4.0	68	67	65	88	87	87
8	1.8	1.7	1.7	56	56	58	78	78	80
9	3.0	3.0	3.0	73	74	69	93	92	90
10	3.7	3.5	3.5	65	67	70	89	88	88
Mean	3.4	3.4	3.4	78.6	77.8	73.6	93.6	93.4	92.0

* A—Gaensler-Collins timed vitalometer
† B—McKesson Vitalor, round mouthpiece
‡ C—McKesson Vitalor, oval mouthpiece

testing cost less than $5.00 and have, in the writers' opinion, extended the usefulness of this particular apparatus. It now permits testing the total vital capacity in either the supine, sitting, or standing positions.

Horton and Phillips[2] compared the results obtained with the Vitalor with those obtained with the 9-liter Collins spirometer and obtained marked agreement for the total and the three-second forced expiratory capacities. They could not compare the one-second forced expiratory capacity and maximal expiratory flow rate. In this laboratory, the results obtained by using the Gaensler-Collins timed vitalometer were compared with those obtained by using the modified Vitalor (table 1). There was excellent agreement for all values of the forced vital capacity and the forced expiratory capacities at one second, two seconds, and three seconds when the round metal mouthpiece was used. When the testing was done with the oval mouthpiece supplied by the manufacturer, good agreement was found only in the three-second forced expiratory capacity and in the forced vital capacity, similar to the results obtained by Horton and Phillips.

The breath-actuated transverse movement of the chart was helpful in cutting down repeat maximal exhalations by the patient because the chart was not started on time. It also permitted several graphs to be made on the same chart. Before and after isoproterenol (Isuprel®) inhalation, expirograms could be recorded on the same paper and in the order desired since the initial setting of the paper was exactly where the graph would start.

The plastic ruler expedited the calculation of the forced expiratory capacities by eliminating the tedious counting of squares and the doubtful drawing of the parallel line necessary for the calculation of the maximal expiratory flow rate.

In summary: Modifications are described to the bellows vital capacity apparatus for the testing of total vital capacity, forced vital capacity, forced expiratory capacities, and maximal expiratory flow rate. A plastic ruler is described for easier calculation of the forced expiratory capacities and maximal expiratory flow rates.

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THE SURGICAL TREATMENT OF PATIENTS WITH INFECTIONS OF THE LUNG DUE TO UNCLASSIFIED ACID-FAST BACILLI

While many reports are now published describing various aspects of the problem presented by human infection with unclassified (atypical) acid-fast bacilli, little has been written about the results of surgery. Crow and associates,[1] in a study of sixty-nine patients, referred to thirteen treated surgically and, of these, only seven had resections; three of this group were classed as failures. Lester and co-workers[2] had nine patients treated by lobectomy and noted 33 per cent complications compared with their usual 9 per cent complication rate. In particular, they noted two patients with sputum negative for acid-fast bacilli at and before surgery, and the sputum was positive, again, one year later. The nature of the complications was not described. Chofnas and Newton,[3] in contrast, had noted that those patients with unclassified acid-fast infections tolerated surgical procedures better than was usual with infections with *M. tuberculosis*; but at that time only four of their patients had been subjected to surgery. Florence[4] reported only four patients treated by resection, in all of whom the sputum became negative for acid-fast bacilli; and spread of disease or other complications were avoided. In a recent article, Corpe and Liang[5] suggest that, whenever possible, resectional surgery should be attempted at an early date in the course of lung disease caused by unclassified acid-fast organisms.

This report concerns the surgical treatment of patients with unclassified acid-fast infections of the lung at the North Carolina Sanatorium between 1955 and 1959.

The total number of patients from whom acid-fast organisms were isolated was 75; of these, 21 underwent surgery.

There were 16 white men whose ages ranged from twenty-five to fifty-six, and 5 white women whose ages ranged from twenty-five to forty-four.

On admission, 13 men had moderately advanced pulmonary disease and 3 had far advanced disease; 3 women had far advanced disease, one had moderately advanced, and one had minimal disease. One man had a bilateral resection, making a total of 22 primary operations, The woman with minimal disease was aged twenty-five; she had had scotochromogenic acid-fast bacilli isolated only once from the sputum, had had no acid-fast bacilli in the sputum for five months before surgery, and did not have scotochromogens isolated from her resected specimen, which was a bronchiectatic lower lobe. She is thus rejected from further consideration. All of the other patients had unclassified acid-fast organisms isolated more than once from the sputum, except 2 who had scotochromogens isolated from gastric lavage five times and twice, respectively; the latter had scotochromogens grown from the resected specimen. The distribution of the various kinds (after Runyon[6]) of unclassified acid-fast bacilli admitted in all and the numbers treated surgically are shown in table 1.

Considering the extent and type of disease, this group compares closely with the other surgically treated tuberculous patients in this sanatorium. The differences were that the number of males with unclassified acid-fast infections was greater, comparatively, and the number of Negroes less; and that, in 13 of 19 instances, sputum contained acid-fast bacilli in the three months leading up to resection. Yet, 15 of 16 bronchoscopies showed the mucosa to be healthy, and in only one case was there bronchoscopic evidence of active inflammation.

All of the patients have been followed for at least seven months from the primary operation, and some for as long as four years.

The surgical technique used in this sanatorium has already been described by Floyd and his associates,[7] and will not be repeated here.

Antimicrobial therapy with drugs to which the respective organisms were susceptible was seldom possible. Susceptibility to two drugs was present in only one strain, and complete susceptibility to one drug was present in 4 other strains. Thus, in only one case could advantage be taken of what is usually thought adequate chemotherapy for infections with *M. tuberculosis* var. *hominis*. Nonetheless, all of the operations were done with accompanying therapy of a combination of at least two of the following drugs: streptomycin, isoniazid, PAS, pyrazinamide, or kanamycin.

[1] Crow, H. E., *et al.*: Amer Rev Tuberc, 1957, *75*, 199.

[2] Lester, W., Jr., *et al.*: Trans 17th Conf on Chemother Tuberc, VA—Armed Forces, 1958, p. 289.

[3] Chofnas, I., and Newton, J.: Trans 14th Conf on Chemother Tuberc, VA, Army, & Navy, 1955, p. 205.

[4] Florence, H.: Dis Chest, 1956, *30*, 250.

[5] Corpe, R. F., and Liang, J.: J Thor Cardiov Surg, 1960, *40*, 93.

[6] Runyon, E. H.: Med Clin N Amer, 1959, *43*, 273.

[7] Floyd, R. D., *et al.*: Surg, Gynec, Obstet, 1959, *109*, 467.

The results of the 21 original operations are presented in table 2. The criteria for major complications were the same as those described by Floyd and associates[7]; these patients formed part of their previously reported larger series.

Of the 18 patients who had surgery (one had a bilateral resection), 15 have had wholly satisfactory results. The only complication of the lobectomy series occurred in a 40-year-old white woman who had acid-fast bacilli in the sputum until surgery, far advanced disease, and an obviously inflamed bronchial mucosa seen at bronchoscopy. Fifteen months after the primary operation, she had a thoracoplasty which seems to have taken care of the fistula and resulting space problem, but her sputum remains positive for nonchromogenic acid-fast organisms.

The patient who suffered excessive hemorrhage at and after his segmental resection had a thoracoplasty to close the resulting space, but has had several hemoptyses, presumably coming from demonstrated bronchiectasis. His sputum has been negative for acid-fast bacilli sixteen months postoperatively, and he has given no indication of new disease.

Two patients who had segmental resections without acid-fast bacilli in the sputum preoperatively developed bronchopleural fistulas. Quite unexpectedly, in the first patient, at one year a small roentgenographic density suggesting new disease appeared and a fistula developed. A further period of bed rest and chemotherapy was followed by a thoracoplasty, and the patient has been well and working for one year now. The second patient was operated on within six months of roentgenographic evidence of spread of disease to the other side, and his bronchus did not close in the postoperative phase. After an extensive thoracoplasty, and despite a subscapular space infection, his disease seems to be controlled.

Both primary thoracoplasties were failures. One was in a 51-year-old man with far advanced, bilateral disease, sputum positive for acid-fast bacilli, and poor respiratory function. An autopsy was not permitted, and it was thought that he died of respiratory failure. The second was in a 41-year-old woman also with far advanced, bilateral disease, acid-fast bacilli in the sputum, and persistent cavitation. Her cavity did not close, her sputum remained positive for acid-fast bacilli, and nine months later she had a lobectomy under the thoracoplasty as it was still too early to assess the results of the second operation.

TABLE 1

DISTRIBUTION OF UNCLASSIFIED ACID-FAST BACILLI IN PATIENTS AND THE NUMBER OF PATIENTS TREATED SURGICALLY

	Nonchromogens	Scotochromogens	Photochromogens
Total patients with unclassified mycobacteria	43	21	11
Patients treated surgically	12	3	5

TABLE 2

RESULTS OF ORIGINAL SURGERY

Type of Operation and Presence of Acid-Fast Bacilli in Sputum	Number	Complications and Nature	Operative Deaths	Sputum Positive for Acid-fast Bacilli between Surgery and Follow-up	Well at 7 Months and Afterward
Lobectomies—bacilli present	9	1 bronchopleural fistula	0	1	8
Lobectomies—bacilli absent	1	0	0	0	1
Segmental resections—bacilli present	4	1 excessive hemorrhage at and after surgery	0	0	3—1 man has minimal hemoptysis
Segmental resections—bacilli absent	5	1 bronchopleural fistula at one year; 1 postoperative bronchopleural fistula	0	0	4—1 man has a healing subscapular space infection
Total resections	19	4	0	1	16
Thoracoplasty—bacilli present	2	0	1	1	0

All of the resected specimens were examined and in each case the appearances were similar to what would be expected in a comparable case of infection with *M. tuberculosis*. The usual report was: "Typical appearance of tuberculosis." Unclassified acid-fast organisms were grown on culture of 14 of the 19 resected specimens and, in each instance, the cultural characteristics were those noted before surgery.

* * *

While it is yet too early to judge final results, it would seem, on reviewing this series of surgically treated patients, that the early results of surgery and the complication rate compare favorably with the results for similar patients with tuberculous infections treated in the same institution at the same time. This is so, despite the high percentage of patients who had acid-fast bacilli in the sputum at surgery (13 of 19 resections), and the small number (one of 19) who had adequate chemotherapy.

These findings agree with those of Corpe and Liang[5] rather than Crow and associates[1] or Lester and his colleagues.[2]

While the numbers are too small for accurate comparison, it may be that complete lobectomy is a safer procedure than segmental resection. The only bronchopleural fistula in this series of 9 lobectomies would have been avoided if the bronchoscopic appearance had been regarded as a contraindication.

There were no deaths in the resected group and, in 12 of 13 patients, the acid-fast bacilli have disappeared from the sputum. This result compares favorably with the results in patients treated pre-surgically on this service, with 5 deaths due directly to unclassified acid-fast infections among 54 patients twelve days, four months, sixteen months, two years, and four years after admission. Of only 12 of 49 patients treated in the sanatorium for six months or longer have the acid-fast bacilli disappeared from the sputum.

It would seem that it is safe and proper to resect either by segmental resection—or, preferably, lobectomy—segments of lung diseased with unclassified acid-fast organisms, particularly when confined to one lobe, despite the absence of adequate chemotherapy and even in the presence of acid-fast bacilli in the sputum. On the other hand, it is probably wrong to cut through a bronchus shown to be diseased at bronchoscopy.

In summary: The results of surgical treatment of 21 of 75 patients with pulmonary infections due to unclassified acid-fast bacilli are presented and it is shown that, despite the absence of adequate chemotherapy and in the presence of acid-fast bacilli in the sputum, the outcome is usually satisfactory, particularly for lobectomies. Two thoracoplasties were unsuccessful, both in patients with far advanced disease.

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September 6, 1960

[6] The writer's thanks are due to Drs. W. H. Gentry, Associate Superintendent—Medical Director, North Carolina Sanatorium, McCain, and W. H. Hollister, Thoracic Surgeon, Pinehurst, North Carolina, for their advice and permission to review these cases.

Correspondence

SICKLE CELL TRAIT AND TUBERCULOSIS

To the Editor of the American Review of Respiratory Diseases:

The problem to be resolved is whether or not the sickle cell trait is one of the factors predisposing the Negro to tuberculosis. The solution has been sought through statistical analysis of data on the sickle cell trait in tuberculous Negroes compared with that from another nontuberculous population. Although statistical analysis may be performed in different ways, it is usually the collection of data, which may be subject to conscious or unconscious bias, which is responsible for any discrepancies that may arise.

In Dr. Weiss's study, the diagnosis of sickle cell trait was made on the basis of clinical data and the results of the sickle cell test. Dr. Weiss, himself, comments on the vagaries of the technique of sickle cell preparation,[1] and we have found it to be capricious from time to time. For screening to be satisfactory, this test must be supplemented by electrophoretic analysis of hemoglobin. This was done in a study by Lyons,[2] whose results we have confirmed. Data on the incidence of sickle cell trait which are obtained from a relatively small series on the basis of the sickle cell preparation alone must be accepted with caution.

Since the sickle cell trait is an hereditary characteristic, its incidence may vary from area to area and may be different even in different segments of what appears to be a homogeneous population group. Dr. Weiss selected his tuberculous patients *at random* from the wards, but his controls were *consecutive* nontuberculous hospital admissions. Our tuberculous patients, the entire population of two tuberculosis hospitals studied, were compared not only with a large series of consecutive prenatal patients collected over a fifteen-month period, but also with a group of more than 300 consecutively studied Negroes who appeared for pre-employment physical examinations at D.C. General Hospital. No significant difference was found.

If the sickle cell trait predisposed to illness, its incidence in the control population should be reduced, accentuating the difference between the control group and the higher incidence in a tuberculous group. This was not found.

As to the type of tuberculosis observed in patients with the sickle cell trait as compared to those without the trait, we studied not only the extent of the disease, as Dr. Weiss indicated in his letter, but also the character of the lesions. As we stated in our report, "When examined as to age, extent, and *type* of disease, there was no significant difference between the two groups." Of course, when the type of disease was evaluated, not only were the roentgenographic characteristics of the lesion appraised but also the clinical manifestations.

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September 20, 1960

[1] Weiss, W.: AMA Arch Intern Med, 1955, *96*, 833.

[2] Lyons, H. A.: AMA Arch Intern Med, 1955, *96*, 833.

Books

CLINICAL EVALUATION OF NEW DRUGS. Edited by S. O. Waife and Alvin P. Shapiro. Pp. 223, Hoeber-Harper Inc., New York, 1959. Price, $7.50.

This collection is timely because the importance of clinical pharmacology is beginning to be recognized, not only by the pharmacologist and all prescribing physicians, but in areas of government. The book's many diverse chapters give an over-all view of clinical research which is an excellent beginning source for anyone involved or interested in the field. It suffers from unevenness in the quality of the writing from chapter to chapter.

The rapid production of new compounds by the chemists and the pressures to have these evaluated for human disease are greater each year. It has not been the tradition in American medicine to produce physicians who are trained pharmacologists, yet a significant amount of work undertaken to test compounds in humans is done by physicians occupied with their daily practice. Here is a general presentation of the problems associated with pre-clinical and clinical pharmacology. The adequacy of animal data, before human studies are undertaken, will always be open for discussion and will vary from compound to compound depending upon its anticipated use. Dr. Karl Beyer, in Chapter 2, mentions that it may not be desirable to give doses sufficient to kill the animal. His reasons for this are acceptable, but the doses should be large enough to produce signs or symptoms of toxicity. It is not too difficult to distinguish between inanition or other side effects if multiple doses are used and the higher dose is designed to produce toxicity. To avoid the initial toxicity in humans, we perform the animal studies, not to give us a feeling of security, but in hopes that these experiments will help us to anticipate the human reactions.

Active compounds are the over-all objective involved here. One of the many pitfalls in this search is the placebo reactor. Dr. Henry Beecher covers both therapeutic and toxic effects which may be described by these individuals, but also lets us know that at present we have no way of selecting the placebo reactor before the study.

Dr. William Bean successfully and briefly poses the moral issue of human experimentation. His views and this problem should be reviewed frequently by anyone directly or indirectly involved in human trials. Human moral fiber, as he recalls, is not always difficult to distort and, when short cuts in human pharmacology are attempted, disaster may be expected to follow.

Another area of difficulty in evaluating the clinical pharmacology of compounds relates to the type of controls that will be utilized in the study. Dr. Harry Dowling discusses this problem with reference to infectious diseases. This reviewer feels that he does not stress sufficiently the confusion that can result with such attempts in controls based on previous years or on other investigators' series. Present hospital-care facilities differ from those of previous years, and vary from one hospital to another to such an extent that direct comparisons cannot be made of single therapeutic agents on this basis. Previous controls are not possible in acute severe infections. The use of alternate case controls, with the unknown medicine being compared with a known standard at that time, affords a much more direct comparison.

The final chapter of the book is a worthwhile discussion of the problems involved in transmitting the results to others. Scientific studies should be undertaken with the plan that worthwhile findings be made available to all individuals in the field. An informative accurate communiqué is difficult but mandatory to allow a comparison with other workers.

Whether or not he desires it, every physician is involved in the experiment of testing compounds against human diseases. Most will find in this book much that is of interest and assistance.

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CLINICAL AUSCULTATION OF THE HEART. By Samuel A. Levine and W. Proctor Harvey. Pp. 657, W. B. Saunders Company, Philadelphia and London, 1959. Price, $11.00.

The development of new techniques in cardiovascular diagnosis has not led to the decline of the stethoscope but, rather, to an increasing appreciation of the value of thoughtful auscultation. In this second edition of a well-known text, the authors again convincingly document the thesis that most disorders of the heart can be accurately diagnosed by simple means.

Doctors Levine and Harvey have emphasized bedside diagnosis; phonocardiography is introduced to demonstrate findings, but is always pre-

sented as a supplement to the bedside findings. Following a discussion of the techniques of auscultation, including the identification of sounds, the separation of hemodynamic events, and the reinforcement of faint murmurs, the authors present an excellent account of the genesis and significance of the heart sounds. The effects of the respiratory cycle on the splitting of heart sounds, systolic ejection clicks, and diastolic gallops are among the topics lucidly discussed.

A description of the findings in acquired and congenital heart disease follows. Differential points are emphasized, common pitfalls pointed out, and brief case histories frequently related. In the section on patent ductus arteriosus, for example, the typical continuous murmur "enveloping the second sound" is described, atypical findings in infancy and in those with pulmonary hypertension are outlined, and conditions also producing continuous murmurs, such as perforation of a sinus of Valsalva, truncus arteriosus, and pulmonary A-V fistula, are considered.

This is a basic book written by experts. Always pleasant and informative reading, it is recommended to all physicians and medical students interested in intelligent bedside cardiovascular diagnosis.

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Recent Advances in Respiratory Tuberculosis. Fifth edition. By F. Heaf and N. Lloyd Rusby. Pp. 284, Little, Brown and Company, Boston; and J. & A. Churchill, Ltd., London, 1959. Price, $8.00.

This new edition presents a comprehensive yet compact review of the numerous advances that have taken place in the diagnosis, therapy, and epidemiologic control of tuberculosis in the past ten years. It is a very well-written textbook, with good bibliographies appended to each chapter. Interested readers have the advantage of gaining a concise review of various controversial issues as well as availing themselves of the more extensive literature via the excellent bibliography.

The authors have provided an adequate evaluation of epidemiology, prevention, tuberculin and tuberculin testing, BCG vaccination, chemotherapy, collapse therapy, resectional therapy, primary tuberculosis, respiratory function studies, chest roentgenography, rehabilitation, miscellaneous advances, definitions, classifications, statistics, and recent legislation. Because the book presents a well-documented, panoramic view of tuberculosis, this reviewer does not hesitate to recommend it to students and practicing physicians, as well as to those engaged in teaching the subject.

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Diagnostic et Traitement de la Tuberculose Pulmonaire. By J. Centner. Pp. 235, Éditions Arscia S. A., Bruxelles, Belgium, 1959.

The specialist does not always appreciate the difficulties which face the general practitioner in following the evolution of knowledge and ideas in phthisiology. The numerous advances in recent years, published in specialized journals, too often escape the notice of those who need to apply them daily.

This book, by the medical director of the Sanatorium of Mont-sur-Meuse (Belgium), is a résumé of present practical ideas in the diagnosis and treatment of pulmonary tuberculosis. According to the author, "it is addressed to the medical practitioner who no longer has the time to encumber himself with theories, and to the student who wishes to put into practice the theories he has learned."

The first part of the work is given to a description of diagnostic methods and to a critical study of the knowledge thus obtained. Here one finds set forth with simplicity the data of the clinical examination, an analysis of the elementary roentgenographic pictures of tuberculosis, the technique for sputum examination, the method for culturing and determining the susceptibility of tubercle bacilli to antimicrobials, and the techniques for performing tuberculin tests and bronchoscopic and bronchographic procedures.

The author has neglected no practical aspect of the diagnosis of tuberculosis, and each chapter contains a methodic and precise description of the techniques to be used, the requisite material, and the interpretation of the results. Certain details may appear elementary to the informed specialist, but one appreciates the necessity of a clear exposition for those with insufficient experience.

A schematic synthesis of the elements of diagnosis of the various forms of pulmonary tuberculosis closes this part of the book.

The second part of the study deals with the treatment of pulmonary tuberculosis. The author observes the same rules of simplicity in his discussion of this subject. He stresses the need for treatment by combined antimicrobials continued for a long duration and the necessity for attentive roentgenographic and biologic supervision. He explains the various techniques of medical and surgical collapse therapy, pointing out the indications for such treatment, which today are some-

what limited. He gives an important place to excisional surgery, its indications, and postoperative supervision.

One cannot sum up this study in a few lines, as conciseness and a schematic format are its principal qualities. The author has voluntarily excluded the bibliographic references which would have given weight to his exposition. On the other hand, he has illustrated his book with numerous roentgenographic reproductions, with diagrams and photographs which facilitate comprehension of the text for readers who are not fully initiated in pneumologic techniques.

This work is a remarkably practical treatise which will be of great value to students and practicing doctors. It also has the merit of bringing to us the practical experience of a sanatorium doctor who is faced with the fundamental problems of the treatment of pulmonary tuberculosis and who, with sound knowledge, has equipped and organized the establishment he directs, adapting it to the necessities of modern phthisiology.

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Lille, France

Rak Legkogo. Klinika, Diagnostika, Lechenie (Cancer of the Lung. Clinical Aspects, Diagnosis, and Treatment) (in Russian). By F. G. Uglov. Pp. 357, Medgiz, Leningrad Branch, Russia, 1958. Price, 20 rubles, 35 kopeks.

The author states in the preface that the literature of the past is very poor in the diagnostic approach to the discovery of early cancer of the lung. The book, therefore, is a guide to students and general practitioners. It is well written, lucid, up to date, based on the author's personal experience as well as on the world literature on the subject. The literature from the United States is liberally referred to; Ochsner and Overholt and many others are frequently quoted.

The eighteen chapters cover the subject fully. Three chapters are devoted to surgery and complications. Unfortunately, nothing in the book deals with anesthesia. This is a serious omission, particularly since the book is to serve as a guide to physicians over the vast land of Russia and since anesthesia is such an important factor in the immediate operative morbidity and mortality. Anesthesia deserves a full chapter and it would make the book a complete treatise.

The chapter on special diagnostic procedures discusses the universally accepted methods such as bronchoscopy, biopsy, bronchial washings, et cetera. Considerable space is devoted to a hematologic method of differential diagnosis. Paucity of granules in the cytoplasm of neutrophils and thrombocytosis of old thrombocytes are frequently found in the blood in the presence of carcinoma.

The reproductions of the roentgenograms are poor, which is due to the poor quality of paper used; the same paper is used for the reproductions as for the text. The colored plates of cytologic studies of specimens are very good, and are on glazed paper.

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BOOKS RECEIVED

Diseases of the Newborn. By Alexander J. Schaffer. Pp. 878, W. B. Saunders Company, Philadelphia, and London, 1960. Price, $20.00.

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Collected Papers of the Mayo Clinic and the Mayo Foundation, Volume 51, 1959. Edited by Carl M. Gambill *et al.* Pp. 825, W. B. Saunders Company, Philadelphia and London, 1960. Price, $14.00.

The Multilingual Manual for Medical Interpreting. By Louis R. M. Del Guercio. Pp. 160, Pacific Printing Company, New York, 1960. Price, $2.50.

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NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1961—Cincinnati, Ohio—May 21-25
1962—Miami Beach, Florida—May 20-25
1963—Denver, Colorado—May 12-17
1964—New York, New York—May 24-29

MEDICO-SURGICAL CINEMA

The annual prize for medico-surgical cinema, endowed with 1,000 NF in cash (which, if necessary, can be divided) and various other awards, will be presented during the last session of the course in Actualités Médico Chirurgicales at the Nouvelle Faculté de Médecine de Paris on March 14, 1961.

The jury will consider the didactic value of the films submitted as well as their cinegraphic quality. Only 16-mm. films will be eligible, and projection time must not exceed thirty minutes.

Entries (in the form of the films themselves) are to be sent to the Secretariat du Journal *La Presse Medicale*, 120 Boulevard Saint-Germain, Paris VI°, prior to February 1, 1961. Entries may include films subsidized or produced by laboratories or firms.

CHARTER MEMBERSHIP IN THE AMERICAN SOCIETY OF DIAGNOSTIC RADIOLOGY

Applications for charter membership in the American Society of Diagnostic Radiology are now being received. Membership is open to cardiologists, chest physicians, gastro-enterologists, rheumatologists, orthopedists, pediatricians, otolaryngologists, internists, and general practitioners who do or may desire to do some types of diagnostic radiology in their offices.

For further information, write to: Dr. Louis Shattuck Baer, 411 Primrose Road, Burlingame, California.

NEWER CONCEPTS OF CARDIORESPIRATORY DISEASE IN CHILDREN
April 13-15, 1961

A 2½-day course in Newer Concepts of Cardiorespiratory Disease in Children, designed primarily for physicians who have not had special pulmonary or cardiac training, is to be held at Tulane University School of Medicine, New Orleans, April 13-15, 1961. The first day will be devoted to respiration, the second to cardiology, and the third (half-day) will consist of application of principles previously discussed to actual patients. The Tulane Faculty will be supplemented by two guest faculty members: Dr. Edward A. Gaensler of the Boston City Hospital and Dr. Patrick Ongley of the Mayo Clinic.

The program will be jointly sponsored by the Tuberculosis Association of Greater New Orleans and the Division of Graduate Medicine of Tulane University. Registration will be limited to 25.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE
Editorial Assistant: TERRI MANN

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The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 42 abstracters systematically review 239 journals which represent 36 countries and which are published in 22 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title.)

ABSTRACTERS

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

The Treatment of Asthmatic Children by Hypnotic Suggestion. J. M. Smith* and C. L. C. Burns. *Brit J Dis Chest*, January, 1960, 54: 78–81. (*Birmingham School Health Service, England)

Hypnotic suggestion on 4 occasions at weekly intervals failed to give any objective improvement, either immediate or delayed, in a series of 25 children between 8 and 15 years of age with chronic asthma. Although little has been written about it, hypnosis has been widely believed to be of value in asthma. The children proved good subjects for hypnosis and the psychiatrist confidently expected good results, having obtained apparently successful results with both adults and children on many occasions during 30 years of practice in psychiatry. The failure to obtain good results may be due to the short duration of the treatment, but even the most superficial form of suggestion is expected to give a percentage of positive results in asthmatics—in the past this has been reported as high as 33 per cent. Objective assessment of treatment in asthma is essential.—M. J. Small

Prolonged Corticosteroid Therapy in Chronic Asthma. A. R. Sommer*, M. C. Rogan, and I. W. B. Grant. *Brit Med J*, April 9, 1960, No. 5179: 1092–1097. (*U. of Edinburgh, Scotland)

An attempt has been made to assess the value of long-term corticosteroid therapy in relieving symptoms in 33 patients with chronic asthma. The assessments, at 4-week intervals, were made clinically and by estimation of forced expiratory volume. Ten patients were given prednisolone on either 2 or 3 consecutive days of each week, and another 10 either on 8 consecutive days per month or on 6 consecutive days each fortnight. These various regimens provided a useful but fluctuating degree of symptomatic improvement. A group of 14 patients received prednisolone or triamcinolone daily for periods varying from 15 months to more than 3 years. A remarkable measure of sustained improvement was obtained in 8 patients. The presence of chronic bronchitis in addition to chronic asthma was believed to be the main reason the other 6 patients failed to show an equally satisfactory response. Serious side effects from corticosteroids were encountered in 4 patients given continuous treatment. Such treatment can be recommended only in exceptional cases in which there is no effective alternative (after authors' summary).—E. A. Riley

Chewing Gum in the Bronchi. F. C. Stallybrass* and R. M. Child. *Brit Med J*, April 9, 1960, No. 5179: 1113. (*Manor Hosp., Walsall, England)

Two sudden deaths are reported from aspiration of chewing gum into the right and left main-stem bronchi. No similar cases have been reported previously.—E. A. Riley

Some Aspects of the So-called Malformed Bronchopneumopathies Due to an Anatomic Defect. Roentgenographic and Clinical Study (in Italian). V. Nitti* and E. Miraglia. *Arch Tisiol*, No. 11, 1959, 14: 1077–1106. (*U. of Naples, Italy)

The clinical, roentgenographic, and bronchographic data obtained in 15 patients, 23 to 59 years of age, with malformed bronchopneumopathies of the upper lobes are given in detail. The processes were more frequently (66 per cent) observed in the right lung, and in 53 per cent all of the branches of the upper bronchi were involved. According to Monaldi's classification of these bronchopneumopathies, the cases studied in this paper were considered caused by defects either of the fourth or of the partial third degree. The clinical picture showed that the first symptoms appeared earlier than 30 years of age in 2 patients, between 30 and 40 in 4, and after the age of 40 or 50 in 9 patients; there was, therefore, a long period without symptoms during which there were no clinical or functional effects. The onset was mostly mild and rather difficult to define. The difficulty of a correct early diagnosis is emphasized and the elements necessary to achieve it are discussed.—I. Archetti

Nitrogen Mustard in the Palliation of Lung Cancer. R. H. Bass*. *Brit Med J*, February 27, 1960, No. 5173: 617–620. (*London Hosp., England)

Twenty-five patients with inoperable lung cancer were treated with nitrogen mustard between 1955 and 1959. The drug was given at night following a light evening meal and was preceded by sedation with sodium amylobarbitone. This drug has proved superior to any other in the prevention of nausea and vomiting. A single dose of 0.4 mg. per kg. was used and repeated every eighth week. Pleural effusions were treated by an intrapleural injection of 15 mg. of nitrogen mustard diluted in 100 ml. of normal saline. This procedure may be followed within 48 hours by a reactionary effusion which may require aspiration. Among the 25 patients, 17 (68 per cent) were improved; 11 of them had anaplastic carcinomas. The longest survival time after treatment was 10 months. Blood counts were taken at weekly intervals after treatment, but no significant leukopenia was noted. There was no evidence that nitrogen mustard prolonged life.—E. A. Riley

"The Coughing Disease" in Children. R. Grenville-Mathers*. *Brit J Dis Chest*, January, 1960, 54: 72–77. (*Edgeware General Hosp., Middlesex, England)

An analysis of 675 children referred to a chest clinic is reported. The majority cleared with simple measures, and only allergic manifestations seemed to persist throughout childhood. It is concluded that coughing is a frequent symptom in children. There is a big increase in incidence upon starting school, and this catarrhal stage seems to persist until age 7, by which time immunity seems to have developed. Direct contact has been shown to be the most important way of spread of respiratory infections; the number of "only" children in this series points to the importance of the school in this spread. Although the clinic developed on the theory that the appropriate treatment of respiratory infections in childhood may prevent chronic bronchitis later, the clearing of these infections by age 7 does not lend support to this view. Great emphasis has been placed on the prevention of pulmonary damage, but the incidence of this damage has been found to be quite high. Although it is thought that a minimal number of cases of bronchiectasis has been diagnosed, these cases gave a rate of 4.1 per 1,000 children roentgenographed. More attention probably needs to be paid to the preventive aspects of coughing in children. Few mothers seem to teach their children the correct way of blowing their noses by clearing one nostril at a time, or to see that they do this frequently. The introduction of breathing and postural exercises, as taught by physiotherapists, into the physical exercise session in the infants' schools would also be a help.—M. J. Small

Palliative Treatment of Malignant Serosal Effusions with Colloidal Radioactive Gold. L. R. Holsti* and A. Salmi. *Ann Chir Gynaec Fenn*, Fasc. 1, 1960, 49: 102–113. (*Sädehoitolaitos, Helsinki, Finland)

During the years 1955–1959, 20 patients with malignant pleural effusions and 16 with malignant peritoneal effusions were treated with colloidal radioactive gold. A single dose ranged from 50 to 150 millicuries. Fifteen patients improved significantly. Some have remained asymptomatic for 1 to 4 years. The best results were obtained in patients with carcinoma of the breast or of the ovary. Patients in poor condition did not benefit from the treatment. The damaging effect of radiation on the free cells in the fluid was clearly shown. Pyknosis and vacuolization were most often observed. Malignant cells were present in the fluid in many cases even after treatment.—J. Haapanen

The Cardiovascular-Respiratory Effects of a New Xanthine Derivative in Chronic Pulmonary Emphysema and in Mitral Stenosis. F. Barrera*, J. C. Dominguez, R. L. Changsut, G. G. Regalado, L. Arias, and J. Faura. *Amer J Med Sci*, April, 1960, 239: 487–491. (*Inst. of Cardiovascular and Thoracic Surg., Havana, Cuba)

Eight cases of advanced pulmonary emphysema and 11 cases of "pure" mitral stenosis were studied during cardiac catheterization, at rest, and 30 minutes after intravenous administration of 0.10 to 0.20 gm. of a new xanthine derivative, 7-(ψ-piperidino-butanon-2-yl-) theophyllin (X-D). The drug increases alveolar ventilation, reduces physiologic dead space, arterial pCO_2, and the physiologic dead space/tidal volume ratio, and increases arterial blood pH. These changes are interpreted as action on pulmonary circulation probably associated with bronchodilatation. The drug produces a drop in systemic and pulmonary pressures at the end of 15 and 30 minutes. It decreases all resistances and increases cardiac output. These changes are due to vasodilatation, both systemic and pulmonary.—W. J. Steininger

Unilateral Emphysema. R. F. Fouché*, J. R. Spears, and C. Ogilvie. *Brit Med J*, April 30, 1960, No. 5182: 1312–1315. (*London Hosp., England)

The clinical, roentgenographic, and physiologic characteristics of 7 patients with increased transradiancy of 1 lung are described. The patients all complained of dyspnea on exertion. Diminished breath sounds and a hyperresonant percussion note were found over the affected lung. All but 1 of the patients gave a history of "pneumonia" in earlier life. Roentgenographic studies revealed impaired expiratory deflation and decreased hilar and peripheral vascular shadows on the affected side. These changes were observed most often in the lower and middle zones of the right lung. The physiologic defect was distinguishable only in degree from that observed in generalized emphysema. The relatively greater impairment of mixing efficiency in the unilateral disease is attributed to a gross disparity in the rate of ventilation of the 2 lungs. The normal values obtained for diffusing capacity in 5 of the 7 patients suggests that the unaffected portions of the pulmonary capillary bed had successfully accommodated the increased blood flow diverted to them. In 1 patient studied by bronchospirometry, hyperventilation resulted in overinflation and temporary suspension of oxygen uptake on the affected side. It is suggested that transient bronchial obstruction in the course of a

respiratory infection in earlier life may have been responsible for the changes observed in some of these patients (after authors' summary).
—E. A. Riley

Diffuse Interstitial Pulmonary Fibrosis: Report of a Case. A. C. Douglas*. *Brit J Dis Chest*, January, 1960, 54: 86–90. (*U. of Edinburgh, Scotland)

A case is described of diffuse interstitial pulmonary fibrosis with the unusual feature of bone formation in the lesions. The condition was slowly progressive from 1954 until 1958, when steroid therapy was instituted and is being continued with 10 mg. of prednisolone daily. In spite of apparently irreversible histologic changes in the lung biopsy, there has been a most satisfactory response to treatment functionally and roentgenographically.—M. J. Small

Respiratory Obstruction in Haemophilic Patients. R. A. L. Leatherdale*. *Brit Med J*, April 30, 1960, No. 5182: 1316–1320. (*United Oxford Hospitals, England)

Ten cases of nasopharyngeal bleeding occurring in patients with hemophilia are reported in which severe respiratory obstruction occurred. Various methods of relieving the obstruction are discussed.—E. A. Riley

Haemoptysis. R. N. Johnston*, W. Lockhart, R. T. Ritchie, and D. H. Smith. *Brit Med J*, February 27, 1960, No. 5173: 592–595. (*U. of St. Andrews, Scotland)

Among 324 patients with hemoptysis, a cause for the symptom was not found in 145 (44 per cent). Inflammatory conditions of the bronchi, chronic bronchitis, and bronchiectasis accounted for 30 per cent of the total and were more than 7 times as frequent as either bronchial carcinoma or active pulmonary tuberculosis. Calcified primary complexes may render patients more prone to hemoptysis, although the mechanism is in doubt and there is no proof of a causal relation. Systemic hypertension is an uncommon cause of hemoptysis. In carcinoma, hemoptysis tends to be minimal but persistent: a frank hemoptysis is unusual and the certainty of hemoptysis may be in doubt; it is still an important and frequent symptom of this disease. In active pulmonary tuberculosis there is a more brisk bleeding of short duration, and the incidence of this symptom has declined. The amount of bleeding is no index of the underlying pathology. Over a 2-year period, 89.8 per cent of these patients were followed up. So little change was shown that follow-up is not considered necessary, provided the patients are carefully assessed at the first examination. In men older than 40, in whom the risk of carcinoma is higher, bronchoscopy is an essential part of the initial investigation (after authors' summary).—E. A. Riley

Pulmonary Manifestations of Hodgkin's Disease. P. Ellman* and A. J. Bowdler. *Brit J Dis Chest*, January, 1960, 54: 59–71. (*Dorking General Hosp., Surrey, England)

Six cases of Hodgkin's disease are described, illustrating different aspects of pulmonary involvement; 2 showed generalized forms, 3 had essentially localized lesions, and in 1 case the pulmonary features were the only manifestation of the condition when the case presented. The most striking feature of pulmonary involvement in Hodgkin's disease is its remarkable diversity. The roentgenographic appearances of the localized lesions may suggest nodules (varying in size, number, definition, and distribution) which may show coalescence into extensive masses, or there may be massive lobar involvement or confluent lesions involving more than 1 lobe and in continuity with the mediastinal nodes. Peribronchial spread may result in feathery shadows or streaking in the lung fields, while irregular, often poorly defined hilar shadows result from local invasion. The diffuse shadows may be reticular, and these seem to appear earlier in the course of the disease and to be of more benign significance than other pulmonary shadows in this condition. Miliary and "lymphangitic" types of diffuse involvement also occur, and may be virtually indistinguishable from miliary tuberculosis, sarcoidosis, and lymphangitis carcinomatosa. Pulmonary lesions secondary to mediastinal or bronchial disease include atelectasis, massive collapse, or lung abscess, and recurrent infections may follow secondary bronchiectasis.

A wide spectrum of pulmonary expression may be present in Hodgkin's disease, and it may well be that these manifestations will become more prominent, if not more common, as advances in therapy improve the prospects of survival in this disease. If so, problems arising in their recognition and management will require increasing attention, since so often in Hodgkin's disease the well-being of the patient depends on careful appraisal of secondary and sometimes apparently marginal features of his condition.—M. J. Small

Structure of Lymph Nodes in Hyaline Membrane Disease. M. M. Black* and F. D. Speer. *Amer J Clin Path*, April, 1960, 33: 303–309. (*New York Medical College, N. Y., N. Y.)

Eleven of 32 perinatal deaths were the result of hyaline membrane disease. In contrast to fetuses without hyaline membrane disease, the maturation of lymph nodes in those with hyaline membrane

disease was characterized by delay and inadequacy in development of their reticuloendothelial cells. In addition, these nodes tended to have increased prominence of eosinophilic leukocytes. Greater knowledge of vascular homeostasis in the perinatal period may be a requisite for a better understanding of the pathogenesis of hyaline membrane disease.—E. DUNNER

Hydatid Disease in North Wales. O. M. JONATHON. *Brit Med J*, April 23, 1960, No. 5181: 1246–1253.

Among 44 cases of hydatid disease observed in North Wales between 1948 and 1957, there were 13 cases of pulmonary disease. With 2 exceptions, the cysts were unilateral and single. Cough, hemoptysis, chest pain, and dyspnea were the most common complaints. In 2 patients the disease was diagnosed on mass roentgenography. Two patients gave a history of coughing up hydrated "grape-skins" as the result of intrabronchial rupture of the pulmonary cysts, intrapleural rupture occurred in 2 patients, and rib erosion occurred in 2. The Casoni test was positive in 7 of 11 patients tested, and negative in 4. The most common operative procedure was excision of the cysts: lobectomy was performed once only. There was 1 reoccurrence following operation.—E. A. RILEY

Staphylococcal Invasion of Pulmonary Infarcts. B. A. ELLIOT*, G. A. GRESHAM, and D. N. PHEAR. *Brit Med J*, April 30, 1960, No. 5182: 1320–1322. (*Central Middlesex Hosp., London, England)

Four cases are described of pulmonary infarction with superinfection by staphylococci and the development of lung abscesses.—E. A. RILEY

Etiologic Considerations of Bilateral Hilar Lymphadenopathy (in Japanese). R. MIKAMI*, S. YOSHIDA, E. KIRA, E. MIKI, S. MATSUZAKI, Y. MUTO, and T. TAKUMA. *Clin Resp Organs*, May, 1960, 15: 327–340. (*School of Med., Tokyo U., Japan)

The writers tried to diagnose cases of sarcoidosis, which is a rare disease in Japan, by screening roentgenograms showing bilateral hilar lymphadenopathy, as the latter was considered to be an early finding in the disease. Ten cases were selected for etiologic analysis, and the diagnosis was confirmed in only 4 cases. They were 2 cases of hilar lymph node metastasis from gastric cancer, 1 case considered to be due to primary infection of tuberculosis, and 1 case of sarcoidosis. The sarcoidosis was in a 43-year-old female in whom the illness started with right supraclavicular lymphadenopathy, and subcutaneous nodules, uveitis, bilateral hilar lymphadenopathy, negative Mantoux reaction, and increased serum gamma-globulin were demonstrated. The histologic findings from excised lymph nodes were compatible with the diagnosis of sarcoidosis, and stellate inclusion bodies were found in the giant cells of the subcutaneous nodules. The diagnosis in the other 6 cases was not confirmed by histologic examinations, but the symptoms and clinical findings were compatible with the diagnosis of sarcoidosis.—I. TATENO

Myasthenia Gravis with Respiratory Failure Developing after Thymoma Excision. P. D. ROBERTSON*. *Scot Med J*, April, 1960, 5: 162–163. (*Western Infirmary, Glasgow, Scotland)

A woman of 61 complaining of dyspnea on effort was found to have roentgenographic signs of a mass at the right hilum continuous with the heart shadow. Thoracotomy revealed a cystic tumor attached to the thymus. It was removed, and histology proved it to be a thymoma. Eight months after operation the patient developed typical myasthenia gravis. She failed to respond to neostigmine bromide (180 mg. daily) and to pyridostigmine bromide (Mestinon®), and she died in myasthenic crisis. Necropsy revealed only fatty tissue in the remnant of the thymus.—J. LORBER

A Case of Disseminated Histoplasmosis. J. H. O. EARLE*, J. H. HIGHMAN, and E. LOCKEY. *Brit Med J*, February 27, 1960, No. 5173: 607–611. (*Royal Masonic Hosp., London, England)

The fourth case of disseminated histoplasmosis reported in Britain is described. The disease, contracted by the patient in India, was not diagnosed until after post-mortem examination, which revealed extensive involvement of the kidneys and adrenals.— E. A. RILEY

Family Outbreak of Primary Atypical Pneumonia Associated with the Eaton Agent. R. T. JOHNSON*, M. K. COOK, R. M. CHANOCK, and E. L. BUESCHER. *New Engl J Med*, April 21, 1960, 262: 817–819. (*Massachusetts General Hosp., Boston)

The clinical features are described of a family outbreak of primary atypical pneumonia involving 2 adults. The course of pneumonia in both patients was similar in that the initial symptoms were upper respiratory infection with marked fatigue, the development of rales and roentgenographic evidence of pneumonitis were insidious, signs of pneumonia continued long after the febrile course, and convalescence was protracted. Although cold and Streptococcus MG agglutinins developed in only 1 patient, both manifested significant increases in antibody to the Eaton agent as measured by the fluorescent method, and the Eaton agent was isolated from the patient in whom agglutinins failed to develop. This suggests that cold and Streptococcus MG agglutinins are not

specific indices of infection with this agent, and further suggests that the antibody measured by the fluorescence method is indeed specific for the Eaton agent.—M. J. Small

Fulminating and Fatal Pneumonia and Septicaemia Due to *Achromobacter Anitratus*. D. L. Gardner*, H. Pines, and S. M. Stewart. *Brit Med J*, April 9, 1960, No. 5179: 1108–1109. (*U. of Edinburgh, Scotland)

A case is described of pneumonia and septicemia which were fatal within 24 hours. *Achromobacter anitratus* (Schaub and Hauber) Brisou was isolated from the sputum, and a morphologically identical diplococcus was seen in sections of the lung, spleen, and adrenals. Proof that this organism may behave as a pathogen has not been previously established (after authors' summary).
—E. A. Riley

Lipoid Pneumonia with Special Reference to the Genesis and Surgical Significance of Endogenous Lipoid Pneumonia (in Japanese). K. Inada*, Y. Koge, S. Yoshida, Y. Komoto, and M. Hamazaki. *Jap J Thor Surg*, May, 1960, 13: 359–367. (*Okayama U., Japan)

The writers present a report on 7 cases of nonspecific pneumonitis of the cholesterol type. A brief summary of the clinical features simulating lung cancer in some cases and the pathologic characteristics of the disease are described. Histochemical study of lipoid substances was made on surgically removed specimens from several types of chronic pulmonary disease to clarify the origin of the lipoid substances in the lung. Endogenous lipoid was found in almost all types of lesions, although the quantity and location of the substance differed between the different clinical entities. Endogenous lipoid consisted mainly of cholesterol, and was localized intracellularly in cholesterol pneumonitis. It was mainly neutral fat in other chronic pulmonary lesions, although a small amount of phospho- and gluco-lipid were detected histochemically in most cases. After an extensive review of lipid metabolism, the authors emphasize the local origin of lipid substances in cholesterol pneumonitis.—I. Tateno

Leptospirosis Canicola Presenting as Pneumonia. D. H. A. Boyd*. *Brit J Dis Chest*, January, 1960, 54: 91–92. (*Northern General Hosp., Edinburgh, Scotland)

A case is described of presumed human canicola fever presenting as pneumonia, and it is suggested that the pneumonia in this case was due to *Leptospira canicola*. Agglutination reactions for *L. canicola* were positive only to a titer of 1/10, but they were positive to a titer of 1/30,000 ten days later. The patient's work as a miner suggested the possibility of this disease. Treatment with 8 million units of crystalline penicillin daily by injection was curative after failure of treatment with oral chlortetracycline.—M. J. Small

Role of Eaton Agent in Disease of Lower Respiratory Tract. M. K. Cook*, R. M. Chanock, H. H. Fox, R. J. Huebner, E. L. Buescher, and R. T. Johnson. *Brit Med J*, March 26, 1960, No. 5177: 905–911. (*U. S. Public Health Service, Bethesda, Md.)

Considerable serologic evidence for the association of the Eaton agent and viral pneumonia with or without cold agglutinins or Streptococcus MG antibody rises was obtained by use of the indirect fluorescent antibody test. In 2 instances serologic rises to the agent were substantiated by isolation of a strain of the Eaton agent from the sputum of the patient. Antigenic variation and nonspecific reactions could not be demonstrated. Prevalence of fluorescent antibody to the Eaton agent in a general population group suggests that it is a widespread and common infection which may occur in all age groups. Infection with the agent may occur in any month of the year (after authors' summary).—E. A. Riley

Pulmonary Fibrosis in Scleroderma. R. Tranquada*, D. Simmons, and J. Miller. *AMA Arch Intern Med*, April, 1960, 105: 607–612. (*U. of California Med. Center, Los Angeles)

A case is presented of generalized scleroderma with severe pulmonary involvement. Pulmonary function, blood gas, and respiration mechanics studies demonstrated the presence of the alveolar-capillary block syndrome with severe restrictive pulmonary insufficiency, and pathologic correlations of the above are demonstrated. It is emphasized that these changes occurred in the absence of very significant changes in the chest roentgenogram, but were readily detectable through study of the pulmonary functions, lung volumes, and lung and thoracic wall compliances. Compliance determinations are suggested as a technique for early determination of changes in lung and thoracic soft tissues and for objective evaluation of therapy. No beneficial effect of relaxin or corticosteroid therapy was demonstrable (after authors' summary).—T. H. Noehren

Simultaneous Intra- and Extrapulmonary Sequestration. V. Kafa* and V. Beco. *Arch Dis Child*, February, 1960, 35: 51–56. (*Charles U., Prague, Czechoslovakia)

A 3-week-old infant was diagnosed to have left "lower lobe pneumonia" which failed to resolve.

At 11 months of age, a roentgenogram of the chest disclosed a sharply-defined elliptical shadow in the left paravertebral gutter and also below the diaphragm. It was believed to be a retroperitoneal tumor. Operation revealed connecting masses of cysts which were located in the left lower lobe ("intralobar sequestration with an aberrant artery"), passing to the esophagus ("extralobar pulmonary sequestration") and through a hiatus in the diaphragm to the stomach. The infant made a good recovery. Histology confirmed the diagnosis. The literature is reviewed at length.
—J. LORBER

Pulmonary Sequestration. P. TALALAK*. *Arch Dis Child*, February, 1960, 35: 57–65. (*Children's Hosp., Bremen, Germany)

Three cases of pulmonary sequestration are described. An infant of 6 months died immediately after a right lower lobectomy. A cystic malformation of the right lower lobe was supplied by an aberrant artery from the thoracic aorta, and the lesion extended through a diaphragmatic defect into the liver. The second case was in a symptomless boy of 14 with a large dense oval shadow in the right lower lobe, with a fluid level and 2 round protrusions. Bronchography showed distortion of the bronchopulmonary segments. At operation the aberrant artery and the cystic mass were removed with the right lower lobe. The histology was typical. The third patient was an infant of 14 days who was operated on for a large left-sided diaphragmatic hernia. After the abdominal organs had been replaced in the proper position, an atelectatic accessory lobe was discovered which was supplied by an artery arising from the aorta. The lobe was removed and the infant recovered. Histology showed lung structure with bronchiectasis. The theories of pulmonary sequestration are discussed.—J. LORBER

Pulmonary Thromboarteritis Associated with Interatrial Defect and Rheumatic Carditis. S. WILENS* and E. REDFIELD. *Amer J Med*, April, 1960, 28: 631–637. (*New York V.A. Hosp., N. Y., N. Y.)

A case is reported of large interatrial defect, asymptomatic until late in life. The terminal illness was characterized by respiratory difficulties and was due to massive thrombosis of the main pulmonary arteries from the point of bifurcation to their entrance into the lungs. The clinical diagnosis was established by roentgenographic examination of the chest. It is suggested that the lesion in the pulmonary artery was rheumatic in nature, and this is further supported by the finding of characteristic Aschoff nodules in the myocardium and active rheumatic valvulitis of the tricuspid leaflets. It is also suggested that the altered pathway of blood flow through the interatrial defect, changes in pulmonary blood volume and tension, as well as rate of flow, rendered the pulmonary artery vulnerable to this unusually located rheumatic reaction, and that the inflamed vessels were secondarily occluded by thrombi.
—T. H. NOEHREN

Low Sedimentation Rate, Hypofibrinogenemia and Restrictive Pseudo-Obstructive Pulmonary Disease Associated with Trichinosis. E. D. ROBIN*, C. H. CRUMP, and R. J. WAGMAN. *New Engl J Med*, April 14, 1960, 262: 758–761. (*U. of Pittsburgh School of Med., Pennsylvania)

A case of trichinosis and fluctuating eosinophilia is presented in which the diagnosis was considered because of a normal sedimentation rate in the presence of severe inflammatory disease of the muscle. The lack of an elevated sedimentation rate appeared to be based on hypofibrinogenemia. The patient also showed generalized hypoproteinemia, probably on the basis of abnormal liver function. She had dyspnea and tachypnea that appeared to be related to the presence of restrictive pseudo-obstructive pulmonary disease based on invasion of the respiratory muscles by Trichinella larvae. Aberrations of protein metabolism, pulmonary function, and liver function may be useful diagnostic clues in obscure cases of trichinosis (after authors' summary).
—M. J. SMALL

Osteoblastic Bone Metastasis in Gastrointestinal and Bronchial Carcinoids. F. TOOMEY* and B. FELSON. *Amer J Roentgenol*, April, 1960, 83: 709–715. (*U. of Cincinnati, Ohio)

Since the elucidation of the carcinoid syndrome, evidence has been accumulated indicating a close relationship between abdominal carcinoid and bronchial adenoma of the carcinoid type. The present report deals with 2 cases of osteoblastic bone metastases, one arising from an abdominal carcinoid and the other from a bronchial carcinoid. Although bone metastasis in either condition is quite rare, a review of case reports in the literature indicates that a high percentage of those described are osteoblastic in nature. This offers supportive evidence for the thesis that abdominal and bronchial carcinoid are related entities (Authors' summary).—T. H. NOEHREN

Involvement of the Pulmonary Veins by Bronchogenic Carcinoma. K. F. W. HINSON* and H. C. NOHL. *Brit J Dis Chest*, January, 1960, 54: 54–58. (*London Chest Hosp., England)

The methods and results are described of investigations into the frequency of pulmonary vein in-

volvement by bronchogenic carcinoma. In 211 pneumonectomy or lobectomy specimens which had been resected for bronchial carcinoma, the pulmonary veins were opened along their course and sections prepared if definite thrombosis or roughness of the intima in the region of the growth was found. The highest rate was in the oat-cell group (6 of 10), while the squamous-cell tumor showed the least tendency (28.5 per cent). Since it seemed probable that invasion of the vessels as a potential source of malignant emboli was likely to have taken place in the smaller, thinner-walled branches which could not be traced by naked-eye dissection, a special injection method was employed to facilitate the identification of the smaller pulmonary veins. Using this technique it was possible to demonstrate involvement of the tributaries of the pulmonary vein in 40 of the 43 specimens, i.e., in 92 per cent of the cases. It is, therefore, justifiable to assume that some infiltration occurs in all cases. A 3-year follow-up after surgical excision, however, suggests that only infiltration of the major branches has any influence on the prognosis.—M. J. Small

Endobronchial Lipomas (in French). J. Hummel*, A. Roussel, and F. Triboulet. *Sem Hop Paris*, February 18, 1960, 14: 516–521. (*Centre Chirurgical Marie-Lannelongue, Paris, France)

Two cases of endobronchial lipomas are reported and their clinical, endoscopic, anatomic, and pathologic characteristics are discussed. Bronchial lipoma is a rare benign tumor which can only be diagnosed by bronchoscopy. It usually remains undiagnosed for a long time because of lack of characteristic symptoms. These tumors may cause bronchial obstruction and complications which require surgical intervention.—E. Lyon

Pulmonary Hamartochondromas (in Hungarian) G. Mészáros*. *Tuberkulozis*, October, 1959: 224–229. (*U. of Debrecen, Hungary)

This congenital benign tumor may be found in the lung parenchyma or in the bronchi. Its size varies: the endobronchial type is small, the parenchymatous may be large; both may cause stenosis in the bronchi. Diagnosis is made by roentgenography, bronchoscopy, and bronchography. Biopsy must be done to establish the diagnosis and differentiate from other types of coin lesions. Four cases are presented in illustration.
—Z. Virágh

TUBERCULOSIS, PULMONARY

Immunity in Tuberculosis. J. M. Robson* and F. M. Sullivan. *Brit J Dis Chest*, January, 1960, 54: 3–21. (*Guy's Hosp. Med. School, London, England)

Many of the factors involved in the production and mechanism of immunity to tuberculosis are reviewed. Results of experimental work in animals are given in some detail and, when possible, are correlated with observations in man. The results differ somewhat in the details between various species but, in general, a wide range of mycobacteria will protect against tuberculous infection, though the degree of protection varies and at best is never as good as that seen in certain other infections, e.g., diphtheria or smallpox. Immunity develops within a week or so after vaccination, and gradually increases in intensity during the first 2 months. The duration of immunity seems to vary depending on the vaccine, route of vaccination used and, possibly, species, although in man, at least, it seems to last for more than 6 years. The relationship between allergy and immunity is discussed and evidence is given that these are independent phenomena and that either can exist in the absence of the other. Clinically, the importance of this is that failure to convert to Old Tuberculin after BCG vaccination need not mean that good protection has not been developed and, in fact, it is demonstrated that humans can show good protection in the absence of hypersensitivity. The cellular and humoral factors involved in immunity to tuberculosis are discussed, as are also the effects of oxygen tension, local organic acid concentration, and sex.

Both specific and nonspecific factors can modify the resistance to tuberculosis as they do to other infections. Immunity as a rule gives only partial protection, although under favorable circumstances complete inhibition of multiplication of a small inoculum can be achieved. Evidence for the participation of both cellular and humoral mechanisms has been obtained, but there is no doubt that further research on the subject is required.
—M. J. Small

The Use of Aerosols for the Detection of Tubercle Bacilli in Pulmonary Tuberculosis (in Italian). V. Moro* and G. Nai Fovino. *Riv Pat Clin Tuberc*, No. 5, 1959, 32: 559–572. (*Consorzio Provinciale Antitubere., Venezia, Italy)

The authors report the effectiveness of saline aerosol or theophylline-plus-strychnine aerosol for patients with tuberculosis in whom expectoration was entirely lacking or the sputum was scanty and negative. After aerosol, the tubercle bacilli could be more easily detected.
—G. Castelnuovo

Tuberculosis of the Large Bronchi in the Past 14 Years (in French). J. M. Lemoine* and J. Denis. *Rev Tuberc (Par)*, February–March, 1960, 24: 258–266. (*Paris, France)

From 1946 to 1952, 300 cases of endobronchial tuberculosis were diagnosed by bronchoscopy (32 per cent of the patients examined). From 1953 to 1959, only 101 endobronchial lesions were seen (14.4 per cent). Of the 101 lesions, 77 were fibrous stenoses and 24 were ulcerations; the latter were always associated with sputum positive for tubercle bacilli. The lesions were found in the main bronchi in 37 cases, and in the lobar bronchi in the remaining cases; there were 69 lesions of the right and 39 lesions of the left bronchial tree.
—V. LEITES

Apical Localization of Pulmonary Tuberculosis. R. RILEY*. *Indian J Chest Dis*, January, 1960, 2: 1–9. (*Johns Hopkins U., Baltimore, Md.)

There is now convincing evidence that the oxygen tension is ordinarily higher at the apex than in other parts of the lung when a person is in an upright posture. In the horizontal position, the oxygen tension is approximately equal throughout the lung. The initial distribution of tubercle bacilli in the lungs is believed to be random in the case of both blood-borne and air-borne organisms. Studies indicate that primary infections in man are randomly distributed. Reinfection tuberculosis, on the other hand, is characteristically apical. At the time of reinfection, tuberculosis host resistance is higher and the susceptibility of all parts of the lung is, therefore, reduced. In the upright position, however, the higher oxygen tension at the apices makes the upper part of the lung more susceptible than the lower part. As shown by animal studies, people do maintain an upright posture a large enough proportion of the time to render the above-mentioned factors clinically significant.—E. A. ROUFF

Blood Pressure Values in Pulmonary Tuberculosis (in German). G. HASENHÜTTL*. *Wien Med Wschr*, December 12, 1959, 109: 1000–1001. (*Hörgas, Steiermark, Austria)

A fresh tuberculous process induces a lowering of the blood pressure, but the long-standing effect of tuberculous toxin is apt to promote cirrhotic changes in the lungs which in turn cause elevated blood pressure in the lesser circulation. The effect is an increase in the systolic pressure. The blood pressure values were measured in 3 groups of 750 female patients: 299 who had "minimal" tuberculosis registered normal findings; 203 patients with fresh, exudative processes were marked by lower pressure values; those with cirrhotic processes had higher readings. The age of the patients did not markedly influence the findings.
—Z. VIRÁGH

Primary Bronchogenic Carcinoma and Pulmonary Tuberculosis (in Roumanian). C. ANASTASATU*, N. RĂDULESCU, D. CIOFLEC, and M. NEGREA. *Ftiziologia*, November–December, 1959, No. 6: 545–560. (*Clinica Ftiziologică, Timisoara, Roumania)

Tuberculosis preceded the development of lung cancer in 23 patients and the reverse occurred in 10 patients. In all of the cases of cancer observed, an incidence rate of 8.4 per cent is reported regarding the association of these 2 diseases. About ⅔ of the patients were men with an average age of 50 years.—Z. VIRÁGH

Corticosteroid Treatment in Tuberculosis (in German). G. DE CAMP*. *Beitr Klin Tuberk*, November, 1959, 121: 405–446. (*Kladower Damm 221, Berlin-Kladow, Germany)

This is a report on 448 patients—253 with pulmonary tuberculosis—treated with corticosteroids. Only 6 of the 253 became worse. The exudative form of tuberculosis, including pleural effusion, results in the best improvement with this treatment; it should be started as soon as possible. The productive, cirrhotic tuberculous processes are not responsive to the steroids. In 126 patients with bronchogenic carcinoma, the steroid treatment was beneficial at least in promoting the well-being of the patient; it also should be employed postoperatively. In 5 patients with pulmonary abscesses and 20 with sarcoidosis, the favorable results are worth mention. Side effects and complications were infrequent and not serious because the employment of steroid therapy was selective. An extensive bibliography and case histories of several patients are presented.
—Z. VIRÁGH

Echinococcal Cyst and Pulmonary Tuberculosis (in Italian). A. SANI*. *Lotta Tuberc*, No. 12, 1959, 29: 1733–1751. (*Osp. Sanatoriale, Cagliari, Italy)

Short clinical histories are given of 17 patients with both echinococcosis and tuberculosis of the lung. Despite the high frequency of echinococcosis in Sardinia, association of the 2 diseases was found relatively rarely; the coexistence of the 2 processes was accidental and the chronologic relationship was varied. In 7 cases there was no apparent pathogenic relation between the diseases; in the remaining 10 patients a close dependence and influence of echinococcosis on the start, evolution, and final course of the pulmonary tuberculosis could be detected. The prognosis of tuberculosis is serious when it immediately follows either surgical therapy or spontaneous evacuation of the cyst; the prognosis is normal when the tuberculous process has become implanted

upon old residua of echinococcosis. When the 2 diseases are associated and surgical treatment is necessary, it is advisable to prepare and protect the patient with antituberculous drugs.
—I. Archetti

Endoscopic Aspects of Tuberculous Cavities of the Lung. Preliminary Observations (in Italian). G. Babolini*, A. Blasi, and P. Marconi. *Arch Tisiol*, No. 10, 1959, 14: 973–1012. (*U. of Naples, Italy)

When tuberculous cavities of the lung are treated by Monaldi's suction method, it is important to know the true condition of the cavity both before and during the treatment. A new apparatus for cavitary endoscopy is described and the results obtained with it in 20 patients with tuberculous cavities are given in detail for each case. All of the cavities could be observed using this endoscope, except those very small, less than 1.5 cm. in diameter; the color of the cavity walls and the surface conditions could be visualized very well. For a good application of the suction technique the localization of the drainage bronchus is particularly important; this was possible in 60 per cent of the cases.—I. Archetti

Local Treatment of Pleural Tuberculous Exudates with Natrium Succinate of Prednisolone (in Italian). R. Italia*. *G Ital Tuberc*, No. 1, 1960, 14: 32–48. (*U. of Milan, Italy)

Twenty male patients, 20 to 53 years old, with pleural exudates (4 of which were purulent) both primary and secondary to tuberculous processes were treated locally with natrium succinate of prednisolone. The pleural cavity was emptied and 50 mg. of the drug in association with 400 mg. of isoniazid and one million units of penicillin were administered every third day. The purulent cases were treated every day with antimicrobials, and the natrium succinate of prednisolone was given every third day. The blood sedimentation rate, the electrophoretic protein pattern, the antibody titer according to the Middlebrook-Dubos test both in the serum and pleural fluid, the differential leukocyte count in the blood and in the sediment obtained from the pleural fluid, the quantity and quality of the fluid, and the cutaneous tuberculin sensitivity were followed and the various results were analyzed. The clinical outcome of treatment was significantly favorable, especially in 3 cases, 2 of which were secondary to pneumothorax and 1 to discontinuation of pneumothorax.—I. Archetti

The Properdin Level in Pulmonary Tuberculosis (in Italian). A. Callegaro*, E. Gallo, and E. Mazzenga. *Riv Pat Clin Tubere*, No. 5, 1959, 32: 607–613. (*U. of Padova, Italy)

The properdin level was evaluated in 108 patients with various clinical forms of pulmonary tuberculosis. In many cases the properdin level was found to be reduced; it appeared that a certain correlation can be established between the clinical form of the disease and the properdin level. This level was found to be increased in cases of extrapleural pneumothorax.
—G. Castelnuovo

TUBERCULOSIS, NONPULMONARY

Ventricular Aneurysms of Tuberculous Etiology (in Portuguese). J. F. Carneiro* and N. Botelho-Reis. *An Fac Med (Porto Alegre)*, No. 1, 1960, 20: 43–88. (*Faculdade de Medicina, Porto Alegre, Brasil)

It is not customary to cite tuberculosis as a cause of left ventricular aneurysm. However, not less than 9 indisputable cases have been reported, plus 6 others of very probable tuberculous etiology. Caseous lymph nodes in the mediastinum were found in all of the cases. Attention is drawn to the frequency of visceral and lymph node tuberculosis found at post mortem examination of patients without coronary artery occlusion and with ventricular aneurysms of obscure etiology. A frequent but not exclusive localization of such aneurysms is the region of the mirral ring on the postero-lateral surface of the ventricle. Until the present study, the diagnosis of aneurysms of tuberculous etiology was made post mortem. Three cases are presented of calcified ventricular aneurysms in living patients in which the clinical findings, the histories, and the follow-up study allows one to disregard coronary sclerosis and to accept the hypothesis of a tuberculous etiology. Left ventricular aneurysms of any etiology may rupture, causing death; at other times they may persist and eventually show a ring of calcification. These images have often been wrongly interpreted as hydatid cysts of the myocardium. There are cases reported as hydatidosis which are in fact cases of calcified ventricular aneurysm, whether or not of tuberculous etiology (after authors' summary).—J. F. Carneiro

Lupus Vulgaris Cutis in Denmark 1895–1954. Its Relation to the Epidemiology of Other Forms of Tuberculosis. O. Horwitz*. *Acta Tubere Scand*, 1960, 39: Supp. 49, 1–142. (*Finsen Institute, Copenhagen, Denmark)

Extensive analysis is made of 3,902 Danish subjects admitted to the Finsen Institute during the years 1895–1954 with the diagnosis of lupus vul-

garis cutis, an estimated 80 per cent of the cases diagnosed in Denmark. Throughout the present century, the incidence of the disease has been falling sharply: among males, from 25 to 2 per million and among females, from 71 to 4. Age patterns show a sharp peak at age 20 and a more rounded peak at age 50. In the majority of patients the tubercle bacillus found was the human type. Many more deaths than expected took place in childhood and up to the age of 35 years; about ⅓ of the deaths were from tuberculosis. Lupus was the only tuberculous disease in 24 per cent of the patients; 40 per cent had glandular tuberculosis or mucous membrane lupus; 10 to 20 per cent had tuberculosis of the respiratory system, of bone or joints, or other forms of skin tuberculosis. Lupus was the first tuberculous disease in 38 per cent of the patients; it occurred simultaneously in 19 per cent, and after another tuberculous disease in 43 per cent. Within the individual age groups, the incidence of respiratory tuberculosis in patients with lupus as the first tuberculous disease was about 5 times higher than in the general population.—W. J. Steininger

Treatment of Tuberculous Meningitis. J. Lorber*. *Brit Med J*, April 30, 1960, No. 5182: 1309–1312. (*U. of Sheffield, England)

In the course of 12 years, 210 consecutively studied children were admitted and treated for tuberculous meningitis. Of these, 131 (63 per cent) survived, and 105 (50 per cent) have no neurologic or mental sequelae. The results progressively improved with increased experience in management, and with the introduction of PAS followed by isoniazid as specific antituberculous drugs, and PPD and corticosteroids as adjuvants. Since the introduction of isoniazid, 66 of 80 children (83 per cent) survived, and 55 of them have had no sequelae. The most difficult patients are those who are less than 3 years of age and unconscious on admission; to date, none of those have made a complete recovery. Intrathecal streptomycin treatment has not been abandoned, but the minimal course is now reduced to 10 injections. The total duration of treatment is 6 months or longer, but most of this time the children are ambulant and either are treated at home or receive regular schooling in hospital. Systemic corticosteroids are used in children younger than 1 year of age or in those who are unconscious on admission; intrathecal hydrocortisone is of value in threatened or established spinal block (after author's summary).—E. A. Riley

Clinical and Pathogenetic Observations on Tuberculous Meningitis in Children, Considered According to the Unitarian Pathogenetic Concept of Tuberculosis (in Italian). M. Ramaglia* and G. Sampaolo. *Arch Tisiol*, No. 12, 1959, 14: 1169–1217. (*U. of Naples, Italy)

To obtain a better understanding of tuberculous meningitis in children, 120 patients up to 12 years of age who were hospitalized from 1948 to 1958 for tuberculous meningitis were studied for the clinical, pathogenetic, and therapeutic aspects of the disease. All of the cases could be divided into 4 groups corresponding to the various stages of the primary biologic cycle: (*1*) forms of the primary complex (5 per cent); (*2*) forms of the exudative allergic phase (24 per cent); (*3*) forms of the primitive nodular phase (44 per cent); and (*4*) forms of the miliary phase (27 per cent). Complete histories of a few cases are given for each group with a comment on the symptomatology and pathogenesis of the disease. The mortality rate was high (87 per cent) in the first 2 years of life and progressively decreased to about zero per cent in the 7–8th years of age, increasing again between the 8th and 12th years (26–28 per cent). Recovery was obtained in 50 per cent of the patients when they were treated with streptomycin alone, in 69 per cent when streptomycin was associated with PAS, and in 83 per cent when cortisone-like substances were given with streptomycin and isoniazid.—I. Archetti

Diabetes Insipidus after Tuberculous Meningitis. D. R. Hay*. *Brit Med J*, March 5, 1960, No. 5174: 707. (*Christchurch Hosp., New Zealand)

A case of diabetes insipidus is reported occurring in a 3-year-old child after successful treatment of tuberculous meningitis. Only 12 previous cases have been reported, and only 3 cases are recorded in the English literature.—E. A. Riley

Tuberculosis of the Mouth and Throat—with Special Reference to the Incidence and Management since the Introduction of Chemotherapy. R. A. Cawson*. *Brit J Dis Chest*, January, 1960, 54: 40–53. (*King's College Hosp. Med. School, London, England)

Four cases of oral tuberculosis occurring in patients with pulmonary tuberculosis, 1 of primary oral tuberculosis, 8 of tuberculosis of the tonsils, and 1 of lupus vulgaris of the lip, are described. Thirty-four cases of oral tuberculosis associated with pulmonary tuberculosis are reviewed. With few exceptions these patients received chemotherapy, though only for a short period. The features of the various types of tuberculosis of the mouth and throat, including lupus vulgaris, are discussed and the necessity for biopsy examination is emphasized. Five of the cases reported here were followed up for periods from 1 to 10 years

and, with the previous cases reviewed, these show that oral tuberculosis responds rapidly to chemotherapy; but the essential principles of treatment are that several antimicrobials must be given and chemotherapy should be prolonged, usually for 18 months or more. Under these circumstances the danger of hematogenous spread from primary oral tuberculosis is much reduced, and the prognosis of those cases with pulmonary tuberculosis is good (after author's summary).—M. J. Small

Lymphadenectomy in the Treatment of Tuberculous Osteoarthritis of the Lower Extremities (in Roumanian). M. Mihăilescu*, E. Munteanu, C. Lipschütz, and N. Vasiliu. *Ftiziologia*, July–August, 1959, No. 4: 367–372. (*Str. Aristide Briand 7, Bucureşti, Roumania)

Excision of the regional lymph nodes did not influence the course of the disease in 20 patients with tuberculous osteoarthritis and synovitis of the hip or knee joints.—Z. Virágh

Tuberculous Peritonitis. W. Burack* and R. Hollister. *Amer J Med*, April, 1960, 28: 510–523. (*Harvard Med. School, Boston, Mass.)

The bases for this study were the records of 47 patients proved to have tuberculous peritonitis, from the wards of the Harvard Medical Unit of the Boston City Hospital in the past 25 years. Study of the symptomatology and physical findings demonstrated that abdominal pain, fever, or tenderness elsewhere than in the right upper quadrant in a patient who has cirrhosis with ascites should serve to raise the suspicion of coexisting disease, of which tuberculous peritonitis is one possibility. The "doughy abdomen" was found to be a rare and unreliable sign of tuberculous peritonitis. On the contrary, distention of the abdomen with obvious ascites was characteristically present. The absence of physical or roentgenographic evidence of pulmonary tuberculosis was by no means inconsistent with the diagnosis of peritoneal tuberculosis. The ascitic fluid is often physically, chemically, and bacteriologically within normal limits, but when there are more than 2.5 gm. per 100 ml. total protein or more than 250 leukocytes per cu. mm., one should consider tuberculous peritonitis a distinct possibility, particularly when the percentage of polymorphonuclear cells is low. The persistent finding of more than 30 per cent polymorphonuclear leukocytes makes tuberculous peritonitis an unlikely diagnosis. Nearly 1 of every 3 ascitic fluid inoculations into guinea pigs was negative for tuberculosis, and more than half of the attempted mycobacterial cultures were negative. Because either was sometimes positive when the other was negative, there is reason to do both when the disease is suspected. In some cases, exploratory laparotomy was the only means by which it was possible to establish a proper diagnosis within a reasonable time. The two most common reasons for missed diagnoses were: (*1*) not considering its likelihood because the ascitic fluid was "benign," and (*2*) attributing the whole clinical picture to a single diagnosis. —T. H. Noehren

Pott's Disease and Associated Tuberculous Foci. A Retrospective Study of 307 Adults (in French). P. Jacob*, A. Delahaye, P. Treps, and Y. Touzé. *Rev Tuberc (Par)*, February–March, 1960, 24: 213–240. (*Paris, France)

From 1941 to 1956, inclusive, 307 patients with 339 active tuberculous lesions of the spine associated with other tuberculous foci were treated at the Hospital Center Emile Roux in Paris; 159 of the 307 received antituberculous chemotherapy: streptomycin–PAS (82), isoniazid–streptomycin–PAS (72), isoniazid–PAS (5). At the time of admission, 29 patients had paraplegia caused by the spinal lesions. Chest roentgenograms showed normal parenchyma in 20, active pulmonary tuberculosis in 231 (123 with cavitation), old healed tuberculosis in 54, and active endobronchial tuberculosis in 2 patients. There were 8 cases of pleural effusion at the time of admission. In addition, 68 patients gave histories of pleural effusion in the past; thus, the total incidence of pleural effusions, past and present, was 25 per cent. The total number of associated tuberculous lesions in the patients' past histories as well as during hospitalization was 578: 92 were tuberculous lesions of the bones and joints, 287 were pulmonary and endobronchial lesions, 76 were pleural effusions, and 123 were varied visceral lesions. All of the patients were treated with immobilization during the active phase of their illness. Fusion was performed in most cases and was done at the beginning of recalcification; antimicrobial therapy made it possible to perform the operation at a much earlier time. Among the 148 patients treated before antituberculous chemotherapy, 38 died of tuberculosis of various organs; there were 17 deaths among 159 treated with chemotherapy. Of 212 patients with foci treated with spinal fusion, 11 were not cured at the time of discharge; 131 showed anterior pseudoarthrosis and posterior ankylosis; 70 showed anterior and posterior ankylosis. Long term follow-up was obtained in 120 cases: 13 rehospitalizations for tuberculosis, 7 with Pott's disease and previous fusion; of 24 deaths, the cause was not known in 15, and was due to tuberculosis in 6; of 83 patients followed from 1–16 years, 62 were working; those not working had inactive Pott's disease and inactive pulmonary lesions. —V. Leites

Tuberculosis of the Salivary Glands (in Italian). F. D'Aiutolo* and L. Abate. *Riv Pat Clin Tuberc*, No. 3, 1959, 32: 386–395. (*Ist. C. Pizzardi, Bologna, Italy)

Two cases of tuberculosis of the salivary glands, one monolateral and one bilateral, are described in detail. The possible pathogenesis of this form of tuberculosis is discussed.—G. Castelnuovo

Pulmonary Tuberculoma (in Italian). C. Mazzini* and A. Pisanu. *Riv Pat Clin Tuberc*, No. 3, 1959, 32: 396–431. (*U. of Genova, Italy)

This paper deals with the definition of pulmonary tuberculoma, its pathogenesis, anatomic and histologic characteristics, diagnosis, and treatment. The authors describe the observations made on 45 cases of pulmonary tuberculoma. They suggest that treatment should consist of adequate chemotherapy and long-term sanatorium supervision. A long-lasting stabilization of the caseous forms of tuberculomas is possible with this treatment.—G. Castelnuovo

UPPER RESPIRATORY TRACT DISEASE, VIRAL

Viruses, Bacteria, and Respiratory Disease in Children. P. S. Gardener*, J. P. Stanfield, A. E. Wright, S. D. M. Court, and C. A. Green. *Brit Med J*, April 9, 1960, No. 5179: 1077–1080. (*King's College, Newcastle upon Tyne, England)

Among 146 children with acute respiratory infections, there were 28 with upper respiratory infections, 6 with croup, 37 with acute bronchitis, 27 with acute bronchiolitis, and 48 with bronchopneumonia or segmental pneumonia. Except in the patients with acute bronchiolitis, an assortment of viruses was found in each clinical group, but no virus predominated. Among the children with acute bronchiolitis, no virus agent was identified either culturally or serologically. Evidence of a virus was found in 11 of 28 cases of upper respiratory infections, in 14 of 48 cases of pneumonia, and in 5 of 37 cases of acute bronchitis. Bacteriologic investigation revealed a variety of organisms, many of which are normal inhabitants of the respiratory tract, and the isolation rates of a potential pathogen such as *H. influenzae* were in the same order as have been obtained in surveys of normal children.—E. A. Riley

Trial of Adenovirus Vaccine in Royal Air Force Recruits. J. S. Wilson*, P. G. Grant, D. L. Miller, C. E. D. Taylor, and J. C. McDonald. *Brit Med J*, April 9, 1960, No. 5179: 1081–1083. (*Central Public Health Lab., Colindale, London, England)

A limited supply of commercially prepared trivalent adenovirus vaccine containing strains of types 3, 4, and 7 was administered to 402 R.A.F. recruits to test its value. Two of 5 flights of recruits arriving at Bridgnorth each week for 3 weeks during February, 1959, were inoculated. Influenza A, influenza B, and adenovirus infections were all highly prevalent at the time the injections were given and during the weeks that followed. The adenovirus strains isolated were predominantly type 3. There was a total reduction of 40 per cent in the admission rate for acute respiratory illness occurring in the inoculated recruits more than 1 week after inoculation. The protective effect of the vaccine against adenovirus infection, estimated from virus isolation results, was 70 per cent (after authors' summary).
—E. A. Riley

THORACIC SURGERY

Endocavitary Aspiration in the Light of Modern Antituberculous Therapy (in Italian). F. Jacometta Longarato*. *Riv Pat Clin Tuberc*, No. 5, 1959, 32: Suppl., 82–87. (*Osp. Sanat. dell'INPS, Padova, Italy)

Endocavitary aspiration must be considered, according to the author, as an alternative to resection, as a therapeutic preparation for resection, as an autonomous therapeutic treatment or associated with other treatment in post-exeresis relapses, or as an alternative to exeresis.
—G. Castelnuovo

Bronchiectasis. I. Clinical Features and Surgical Treatment. O. Peräsalo*, T. M. Scheinin, and P. Pantzar. *Ann Chir Gynaec Fenn*, Fasc. 1, 1960, 49: 31–50. (*Kirurgi, Helsinki, Finland)

During a 20-year-period, 215 of 416 patients (52 per cent) with bronchiectasis were treated surgically; most of them were less than 40 years of age. Preoperative drainage and antimicrobial treatment improved the results significantly. The bronchiectasis was bilateral in ⅓ of the patients, but bilateral resection was performed in only 12 cases. The mortality and the incidence of complications had decreased due to a decrease in infectious complications.—J. Haapanen

Primary Bronchogenic Carcinoma (in Roumanian). L. Daniello*, C. Papahagi, L. Hica, V. Mihaiu, and A. Şerban. *Ftiziologia*, September–October, 1959, No. 5: 451–458. (*Clinica de Ftiziologie, Cluj, Roumania)

Of 452 cases of primary bronchogenic carcinoma observed during the period 1950 to 1956, the diag-

nosis was made by bronchoscopy in 336 patients, by thoracotomy in 25, by transthoracic puncture in 2, and by biopsy of metastatic lesions in 13. The disease was more frequent in males, more prevalent in urban areas. The average time elapsed from onset to diagnosis was about 10 months. Surgical treatment—with a survival time of more than 4 years—was successful in 14 patients (7 per cent).—Z. VIRÁGH

Traumatic Chylothorax in a Closed Chest Injury: Report of a Case. D. REA*. *Brit J Dis Chest*, January, 1960, 54: 82–85. (*Queen Mary's (Roehampton) Hosp., England)

A report is given of a case of traumatic chylothorax following a closed chest injury. An initial latent period when the patient suffers only from the direct effect of his injury is characteristic. Chest films taken at this stage show no effusion. The average latent period is 12 days; in this case it was 4. The presenting symptom is usually dyspnea due to the massive effusion. The effusion is usually right-sided, probably because the only part of its course where the thoracic duct is in contact with the pleura is on the right side. On paracentesis, the fluid produced is a pink creamy color (the chyle being initially blood-stained). Confirmation of its chylous nature is obtained by microscopy. The presence of fat globules and a high protein content are pathognomonic. The mechanism of actual duct injury is difficult to explain. The duct may be stretched over the hyperextended spine, and part of the circumference of the wall is ruptured. Since 1955, ligature of the thoracic duct has become routine in persistent chylothorax, but ligation of the duct should not be considered until frequent aspirations for as long as 2 weeks have failed to produce a remission. After this time there is danger of encapsulation of the collapsed lung. The usual approach is through a right thoracotomy. If the actual leak cannot be found, the cisterna chyli may have to be ligatured.—M. J. SMALL

Oesophageal Diverticula. E. LAUSTELA*. *Ann Chir Gynaec Fenn*, Fasc. 1, 1960, 49: 51–65. (*Kirurgi, Helsinki, Finland)

From 1949 to 1957, 26 esophageal diverticula were treated: 2 pharyngo-esophageal, 19 epibronchial, and 5 epiphrenic. The diagnosis was made by roentgenographic examinations and by esophagoscopy. The most common symptoms were vague or sharp chest pain, dysphagia, vomiting, and belching. Four epibronchial and 4 epiphrenic diverticula were treated by thoracotomy; in 5 cases the diverticulum was excised, in 2 cases invaginated, and in 1 case the esophagus was resected. All of the patients recovered from the surgery. On follow-up study, 4 patients were symptomless, 3 had improved, and 1 felt the condition unchanged, although the diverticulum was roentgenographically reduced in size.
—J. HAAPANEN

Prevention and Treatment of Postoperative Bronchopleural Fistulas (in Spanish). M. SANTOS DE COSSIO*. *Enferm Torax*, 1960, No. 33: 45–53. (*Sanatorio Antituberculoso de Boñar, Spain)

The known principles and measures on the subject are described based on experience in 21 lobectomies, 3 bilateral lobectomies, and 5 pneumonectomies.—Z. VIRÁGH

Late Results of Thoracoplasty and Resection in Pulmonary Tuberculosis (in Spanish). V. ANCICH* and B. MUÑOZ TAPIA, *Rev Chil Enferm Torax*, No. 24, 1959: 5–22. (*Sociedad Chilena de Enfermedades del Tórax y Tuberculosis, Santiago, Chile)

This is a report on a cooperative study conducted by the tuberculosis institutions of Santiago on tuberculous patients operated upon in 1953–54. Thoracoplasty was performed in 306 patients, 2 of them with minimal, 216 with moderately advanced, and 88 with far advanced disease. In 151 patients with a follow-up period of more than 3 years, the results were favorable in 127 patients and unfavorable in 24. Of 23 fatalities, 3 died from nontuberculous causes. Of 255 patients, 7 had pulmonary resection for minimal, 108 for moderately advanced, and 140 for far advanced tuberculosis; in addition, 32 segmental resections, 152 lobectomies, 68 pneumonectomies, and 3 bilobar resections were performed. Complications occurred in 115 patients, 67 of whom developed bronchopleural fistulas or empyema. There was early mortality in 28 (11 per cent), late mortality in 9 (3.5 per cent), and 10 patients (3.9 per cent) died from nontuberculous causes. Of 124 patients with more than 3 years follow-up, the result was favorable in 103 (83.1 per cent). The extent and type of lesions account for the difference in figures as compared with reports from other countries.
—Z. VIRÁGH

ROENTGENOGRAPHY

The Relationship Between Aortic Atherosclerosis and Cancer. W. WINKELSTEIN, JR.*, R. LILIENFELD, J. W. PICKREN, and A. M. LILIENFELD. *Brit J Cancer*, December.

1959, 13: 606–613. (*U. of Buffalo School of Med., N. Y.)

The frequency of atherosclerosis of the aortic and abdominal aorta was determined roentgenographically in a group of patients with cancer and in a group of noncancerous patients. Posteroanterior thoracic roentgenograms are highly specific in indicating the presence of calcifications of the aorta; scout films of the abdomen are somewhat less sensitive, but also highly specific. The frequency of calcification of the thoracic aorta and of the abdominal aorta was essentially the same in both groups of patients. The percentages of cancerous patients with roentgenographic evidence of atherosclerosis of the aorta was essentially the same for the various types and sites of cancer. The previously reported observation that there is a dissociation between atherosclerosis of the aorta in cancerous patients and noncancerous controls was not confirmed in this study. —W. J. Steininger

Study of BCG Vaccination of Mice with the Autoradiographic Method (in French). J.-F. Pasquier* and E. Zajdela. *Ann Inst Pasteur (Par)*, March, 1960, 98: 383–397. (*National Institute of Hygiene, Paris, France)

The autoradiographic technique has been developed and used in combination with the classic Ziehl staining method in mice vaccinated with BCG and secondarily infected with virulent C^{14}-labeled tubercle bacilli. (*1*) Labeled virulent bacilli and BCG bacilli have been differentiated with this technique in the organs of mice. (*2*) The development of labeled living tubercle bacilli has been observed in vaccinated animals and controls. (*3*) By analysis of the composition and distribution of the emission of bacilli, the presence of living bacilli could be studied in different aspects: in clumps, isolated, and in the process of multiplication or destruction. By means of emission of C^{14} it was found that, 48 hours after virulent inoculation, tubercle bacilli (mostly nonstainable with the Ziehl method) were present in the lungs and spleens of mice, and multiplied more rapidly and in greater numbers in mice not protected by BCG vaccination. Nevertheless, vaccinated mice showed considerable difference in the degree of histologic lesions, certain autohistoradiographic slides being similar to those in nonvaccinated mice (after authors' summary).—V. Leites

Roentgenographic Study Using Contrast Medium in the Mediastinal Venous System in Silicosis and Silicotuberculosis (in Italian). C. Franchini* and F. Panizzolo. *Ann Med Sondalo*, No. 6, 1959, 7: 331–366. (*Villaggio Sanatoriale, Sondalo, Italy)

In order to study the hemodynamics in the superior vena caval system in silicosis, periodyl, a contrast medium, was injected into 20 patients with various forms of pulmonary silicosis associated with tuberculosis, and 3 or 4 roentgenograms were taken for each examination. The results obtained with phlebography are given in each case together with those of roentgenography without contrast. The venous system showed no opacity at all in 4 cases, little in 5 cases, and was normal in 11 cases; the results in the first 2 groups could be caused by the increased pressure in the vena cava due to the mediastinal sclerosis consequent to silicosis. It can be said from these data that an abnormal picture was found in a rather high percentage of patients (45 per cent).—I. Archetti

Roentgenographic Observations on the Changes Induced by Ronicol in the Pulmonary Pattern (in Italian). M. Lucchesi* and N. Calvi. *Lotta Tuberc*, No. 12, 1959, 29: 1672–1692. (*Consorzio Antitubercolare, Milan, Italy)

Two vasodilator substances, ronicol and ronicol "compositum," both containing nicotinic acid, were given either endovenously, by aerosol, or intramuscularly to 70 mentally ill persons 30 to 65 years old for the purpose of studying the modifications induced by these drugs in the pulmonary vessels and for eventual therapeutic use. Patients with cardiovascular processes were not included. The tolerance of the substances was satisfactory. Of a total of 100 observations, 17 were negative and 33 showed slight changes in the pulmonary pattern. In half of the observations it was quite clear that there was an enhancement of the pulmonary pattern, an increase in the number and diameter of the visible vessels and, sometimes, an increase in the caliber of the large pulmonary veins. There was also an increase in the lung tone, as demonstrated by a slight decrease of tuberculous cavities and a contraction of pulmonary lobes free from the parietal attachment because of the presence of pneumothorax. The best results were obtained with 2 to 3 injections of ronicol compositum. The possibility of using such vasodilator substances in association with antimicrobials in treating pulmonary tuberculosis is considered. —I. Archetti

CHEMOTHERAPY

Contribution to the Knowledge of the Antithyroid Action of Some Drugs with Antituberculous Activity. Studies with I^{131} (in Italian). P. Carenza* and A. Girolami. *Arch Tisiol*, No. 12, 1959, 14: 1218–1230. (*U. of Padua, Italy)

Because of disagreement as to the antithyroid activity of PAS, dihydrostreptomycin, and isoniazid, studies were performed with I^{131} to learn if these antimicrobials possess some action on hormonal secretion, and specifically, the last step of iodine metabolism of the thyroid gland. Wolff's method, based upon the calculation of the speed of the decrease of thyroid radioactivity in rats, was used throughout the experiments. The curves of I^{131} emission were studied in 4 groups of rats, 1 a control group and the others injected subcutaneously with 1 of the 3 antimicrobials, starting 45 hours after the intraperitoneal inoculation of the isotope. The mean values of I^{131} emission were always higher in the drug-treated animals than those obtained in the controls. Only the values in the animals injected with dihydrostreptomycin and isoniazid, however, were significantly greater. In conclusion, dihydrostreptomycin and isoniazid seem to possess a clear inhibitory activity upon the hormonal secretion of the thyroid.
—I. Archetti

Costicosteroids in the Treatment of Tuberculous Children (in German). H. Krukowska*. *Beitr Klin Tuberk*, January, 1960, 121: 523–534. (*Korczaka 5, Otwock, Poland)

In addition to antimicrobial treatment with streptomycin, isoniazid, and PAS, 50 children aged 3½ months to 3½ years were given corticosteroids for 3 to 8 weeks. Three children with pleural effusion were completely cured—without adhesions. All but 1 of 14 children with disseminated exudative disease improved. Of 33 children with paratracheal lymph node tuberculosis with pulmonary involvement, 15 improved considerably, 12 moderately, and in 6 children the treatment was ineffective. Relapse occurred in 4 cases. There were no significant side effects. The tuberculin reaction was observed before and after the administration of the corticosteroids in 27 children: it was larger in 10, decreased in 11, and did not change in 4 cases. In 1 child the negative tuberculin reaction became positive after treatment. Local treatment of various forms of extrapulmonary tuberculosis was almost always successful.—Z. Virágh

Antimicrobial-Steroid Treatment of Active Pulmonary Tuberculosis as a Routine Measure. H. Marcus* and P. Christopoulos. *AMA Arch Intern Med*, April, 1960, 105: 542–559. (*Nathan B. Van Etten Hosp., Valhalla, N.Y.)

Thirty patients with various forms of pulmonary tuberculosis were treated with a combination of steroid hormones and antimicrobial therapy. No patient showed clinical or roentgenographic deterioration during this treatment. All patients showed immediate symptomatic improvement. Clearing observed in serial chest films was prompt, so that at the end of 2 to 4 weeks' observation, only 6 patients showed less than moderate improvement. Twenty-four patients showed moderate, marked, or very marked improvement at 2 to 4 weeks. At the end of 3 months, all but 2 patients showed either moderate, marked, or very marked clearing. This group was compared to a group of 30 patients treated identically except for omission of steroid hormones. The comparison demonstrates the advantages of combination steroid and antimicrobial therapy. Complications were few and mild. On the basis of this experience, it is believed justified to state that combined steroid and antimicrobial therapy is, at present, the best form of treatment for all patients with active pulmonary tuberculosis (after authors' summary).—T. H. Noehren

Neurotoxic Reactions to Cycloserine in the Treatment of Pulmonary Tuberculosis: A Clinical, Electro-encephalographic, and Psychosomatic Study (in Italian). E. Baschieri*, F. Angeleri, and F. Biagiotti. *Riv Pat Clin Tuberc*, No. 1, 1959, 32: 57–77. (*Osp. Sanat. INPS, Arezzo, Italy)

Twenty-two patients with pulmonary tuberculosis were treated for a period of about 4 months with cycloserine. The electro-encephalographic examination showed that this drug produced at times some toxic effects in the central nervous system, but these effects were not severe and were always transitory. In only a few cases was it possible to notice some convulsive manifestations, which eventually disappeared. Therefore, the authors are of the opinion that this drug should be administered, but that it should always be given with care and only under medical control.
—G. Castelnuovo

Treatment of Pulmonary Tuberculosis with Cycloserine (in Italian). C. Sartori*. *Riv Pat Clin Tuberc*, No. 1, 1959, 32: 80–87. (*Ist. C. Pizzardi, Bologna, Italy)

The results obtained in 100 patients with pulmonary tuberculosis who were treated with cycloserine are reported. A remarkable positive improvement in the general condition of the patients was observed in 75 per cent of the cases. Positive roentgenographic changes were noticed in the exudative and recent nodular processes in 35 per cent after treatment with cycloserine for 2–3 months. Poor results were obtained in fibrous cavitary forms in which a contraction of the cavities can sometimes occur.—G. Castelnuovo

Alkaline Phosphatase in the Kidneys, Adrenals, Liver, and Intestines of Guinea Pigs under Normal Conditions and under Cycloserine Administration (in Italian). E. MARINO*. *Riv Pat Clin Tuberc*, No. 4, 1959, 32: 437–447. (*U. of Siena, Italy)

The time of appearance and the distribution of alkaline phosphatase was studied in the various organs of healthy guinea pigs and in guinea pigs receiving large doses of cycloserine. No reduction of alkaline phosphatase appeared when the drug was administered in a daily dosage of 25–50 mg. per kg. of body weight but, when the dose reached 300 mg. per kg. of body weight, a remarkable reduction of the enzyme occurred in the organ parenchymas.—G. CASTELNUOVO

The Therapeutic Action of Cycloserine on Pulmonary Foci of Recent Formation (in Italian). D. ZANNONI*. *Riv Pat Clin Tuberc*, No. 4, 1959, 32: 458–468. (Ist. C. A. Pizzardi, Bologna, Italy)

The action of cycloserine was studied on 20 tuberculous patients with the following forms of the disease: primary, nodular exudative, nodular and recently ulcerated, recent ulcerous lobite, caseous-ulcerous, and recent tuberculous foci in the course of chronic tuberculosis. At clinical and roentgenographic examination after cycloserine treatment, 40 per cent of the specific foci appeared to be cured. The action of this drug seems to have an effect on the absorption of the exudates and on the mesenchymal proliferation and fibrous evolution.—G. CASTELNUOVO

Cycloserine in Chronic Pulmonary Tuberculosis (in Italian). F. CASPANI*. *Riv Pat Clin Tuberc*, No. 4, 1959, 32: 520–537. (*Osp. "S. G. Battista" Alzatz Brianza (Como), Italy)

Thirty-nine patients with chronic pulmonary tuberculosis resistant to other antimicrobials were treated with cycloserine. An improvement in general condition was noted in 64 per cent of the patients, and an improvement on roentgenogram was found in 61 per cent. Cycloserine should, therefore, deserve a place of primary importance, especially in the treatment of chronic tuberculous forms.—G. CASTELNUOVO

Clinical Considerations on Concentrations of Isoniazid in the Blood (in Japanese). T. TOMODA*, Y. AKIYAMA, and T. TSUCHIYA. *Clin Resp Organs*, May, 1960, 15: 367–371. (*Osaka Med. College, Japan)

The concentrations of isoniazid in the blood were determined in 102 cases of pulmonary tuberculosis 4 hours after an oral dose of 5 mg. of the drug per kg. of body weight by the upright diffusion method of Schmiedel, and the following conclusions were obtained. (*1*) The isoniazid blood concentration ranged between less than 0.25γ to more than 2γ per ml., indicating a large individual difference. (*2*) There was no relation between plasma concentrations of isoniazid and liver function. (*3*) There was no relation between duration of administration or the dosage given. (*4*) Improvement of roentgenographic findings and disappearance of tubercle bacilli from the sputum occurred more often in the cases with higher plasma isoniazid concentrations than in those with low concentrations. (*5*) The plasma concentrations of isoniazid and the isoniazid resistance of tubercle bacilli had no correlation with each other. (*6*) Negative peroxidase or catalase reactions of isoniazid-resistant bacilli were seen often in patients with high plasma concentrations of isoniazid.—I. TATENO

The Effect of Isoniazid on the Synthesis of Liver Phosphatides in Guinea Pigs and Rats (in German). E. SCHWARZ* and K. OEFF. *Beitr Klin Tuberk*, December, 1959, 121: 451–454. (*Berlin N 65, Augustenburger Platz 1, Germany)

Isoniazid in nontoxic doses increases the synthesis of phosphatides. This effect corresponds with that of similar lipotropic substances such as choline, cystine, et cetera. The present experiments were carried out by measuring the radioactivity of subcutaneously injected P32, after the animals (previously treated with isoniazid in doses of 50–70 mg. per kg. of body weight for 8–22 days) were sacrificed and bled. Streptomycin possesses a similar faculty, but its significance concerning the antituberculous effect of the drug is not as yet known.—Z. VIRÁGH

Treatment of Tuberculosis with High Dosages of Isoniazid (in French). E. BERNARD*, A. P. JARNIOU, L. ISRAEL, M. ENJALBERT, and CH. FABRE. *Presse Med*, February 6, 1960, 7: 209–210. (*Hospitals Laennec and Perez, Paris, France)

Two groups of tuberculous patients whose cases were more or less identical with regard to extent of disease were submitted to treatment with isoniazid and PAS. One group received isoniazid in a dosage of 15 mg. per kg. of body weight; the other received the routine dosage of 5 mg. Both received PAS in a daily dose of 15 gm. The results were compared at the end of a 3-month treatment period. The roentgenographic improvement was significantly higher in the group treated with the higher dosage; sputum conversion to negative occurred more rapidly in the same group. No ill effects were noted in the group with the high dosage treatment. In only 1 case did treatment have to be discontinued because of nervous dis-

orders, and these symptoms proved reversible. The use of pyridoxine in prevention of nervous disorders, especially in neurotic patients, is under consideration.—E. LYON

Studies on the Treatment of Pulmonary Tuberculosis with Methyl Antigen (in Japanese). A. YOSHIDA*. *Kekkaku*, May, 1960, 35: 345–351. (*Miyagi National Sanatorium, Japan)

Methyl antigen and potassium iodide were given with antituberculous drugs to 48 patients with pulmonary tuberculosis who had definite bacteriologic and roentgenographic findings and histories of long antimicrobial therapy. Methyl antigen was given intramuscularly 8 days per month starting with 0.25 ml. of the dilute and increasing gradually to 1 ml. of undiluted solution. Potassium iodide in a dose of 1 to 3 gm. daily was given orally on the same days. (*1*) Of 48 cases studied, tubercle bacilli disappeared or diminished in number in 25 cases by microscopy and in 20 cases by culture. (*2*) The size of the cavity became smaller in 10 cases, the wall became thinner in 6, and the internal surface of the cavity became smooth in 2, as shown by roentgenography. (*3*) Decreased resistance to antituberculous drugs was shown in many cases, especially resistance to PAS, which was reduced in 23 of 35 cases. (*4*) Blood eosinophilia developed in those who were given methyl antigen with potassium iodide, but not in those patients to whom only the former was given. (*5*) In 4 other patients who had not had chemotherapy previously, sputum became negative early, and marked improvement in roentgenographic findings was observed. From these observations, the author believes that methyl antigen is worth a trial in severe cases of pulmonary tuberculosis.
—I. TATENO

Comparison of Experimental Antituberculous Activity of Thiocarbanidin and Thioamide (in French). F. GRUMBACH* and N. RIST. *Ann Inst Pasteur (Par)*, March, 1960, 98: 373–382. (*Pasteur Institute, Paris, France)

Despite the presence of a common chain (C S N H—) in their molecular pattern, thiocarbanidin (THC) and alpha-ethyl-thioisonicotinamide (1314 Th) have very different action; both are effective against tuberculosis in mice. For abortive treatment of short duration, the minimal active dose of THC is lower than that of 1314 Th; on the other hand, THC has only low activity in curative treatment. In association with isoniazid or 1314 Th, THC does not prevent the emergence of bacilli resistant to either drug. The lack of activity of THC under these conditions seems to be due to poor absorption by the organism (after authors' summary).—V. LEITES

Further Experience with Viomycin, Pyrazinamide and Cycloserine in the Treatment of Pulmonary Tuberculosis. K. BANERGEE* and A. FLETCHER. *Indian J Tuberc*, March, 1960, 7: 67–72. (*Wanless Tb. San., Wanlesswadi, India)

A treatment-failure group of 115 patients were re-treated with either viomycin, pyrazinamide, or cycloserine in combination with isoniazid. In 45 cases, these drugs were used to provide antimicrobial coverage for surgical intervention, with excellent results and a low incidence of operative complications. Among 70 patients who were not suitable for surgical intervention, only 13 had good results. There was one case of hepatotoxicity in the pyrazinamide group, 5 cases of mild or severe psychosis in the cycloserine group, and 1 case of severe drug hypersensitivity in the viomycin group.—E. A. ROUFF

The Medical Letter*. (*136 E. 57th St., N.Y., N.Y.)

July 22, 1960, 2: 57–60: Two new thiazide derivatives, benzydroflumethiazide (Naturetin®) and trichlormethiazide (Naqua®), and the parent compounds: chlorothiazide (Diuril®), hydrochlorothiazide, flumethiazide (Ademol®), and hydroflumethiazide (Saluron®). A new oral anticoagulant, anisindione (Miradon®). Antihistamines for allergic disorders.

August 5, 1960, 2: 61–64: Reappraisal of 3 oral nitrofurans: nitrofurantoin USP (Furadantin®), furazolidone (Furoxone®), and furaltadone (Altafur®).

August 19, 1960, 2: 65–68: Results of the first of a series of tests on drugs to determine uniformity of generic and trade name prescriptions: 5-mg. prednisone tablets. Non-barbiturate sedative-hypnotic drugs: methylparafynol (Dormison®), ethchlorvynol (Placidyl®), and ethinamate (Valmid®). Danger of use of furaltadone (Altafur®).

September 2, 1960, 2: 69–72: Guanethidine (Ismelin®) for hypertension. V. A. study of antihypertensive drugs. Ketostix® and Acetest® tablets in tests for ketonuria.

PULMONARY PHYSIOLOGY

The Alveolar-Capillary Block Syndrome. F. ELDRIDGE*. *AMA Arch Intern Med*, May, 1960, 105: 665–667. (*Stanford U., California)

The term "alveolar-capillary block" syndrome has proved to be a popular one and appears to be a permanent part of the medical nomenclature. Although it is a useful means of identifying the clinical syndrome which often occurs in patients with the varied types of diffuse pulmonary dis-

ease, it should be recognized that the physiologic mechanisms and features of these diseases are multiple and variable and that this identification helps little in the diagnosis or treatment of the primary disease.—T. H. NOEHREN

A Study of Serum Enzymes in Patients Suffering from Periods of Respiratory Insufficiency, Especially Asthma and Emphysema: Preliminary Report. H. COLLDAHL*. *Acta Med Scand*, April 20, 1960, 166: 399–400. (* St. Göran's Hosp., Stockholm, Sweden)

The serum values of such enzymes as glutamic pyruvic transaminase, glutamic oxalecetic transaminase, ornithine carbamyltransferase, and aldolase are often elevated in patients with insufficient exchange of respiratory gases. Very high values in such patients may indicate that they need artificial respiration to normalize the pulmonary gas exchange.—E. DUNNER

Pulmonary Functions Before and After Thoracoplasty Operation and Pulmonary Resection. H. DINGLEY*, K. SINGH, and S. GUPTA. *Indian J Tuberc*, March, 1960, 7: 50–59. (*Tb. Hosp., Mehrauli, Delhi, India)

From July, 1958, to December, 1959, 89 patients were studied preoperatively with respiratory function tests, but in only 47 patients were postoperative studies carried out. There were 37 thoracoplasties, 11 lobectomies, and 4 pneumonectomies in the completely studied group. Studies included vital capacity, maximal breathing capacity, and oxygen intake by conventional means as well as by bronchospirometry. In every case the preoperative values were low, frequently very marked, and quite out of proportion to the extent of the lesion. The mean loss after lobectomy was less than after thoracoplasty. The loss after thoracoplasty increased progressively with the number of ribs removed.—E. A. ROUFF

Some Effects of Restriction of Chest Cage Expansion on Pulmonary Function in Man: An Experimental Study. C. G. CABO*, J. BUTLER, and A. B. DUBOIS. *J Clin Invest*, April, 1960, 39: 573–583. (*Graduate School of Med., U. of Pennsylvania, Philadelphia)

The total lung capacity and its subdivisions were reduced by restricting chest expansion in normal man. The lung pressure-volume relationship was altered over much of the vital capacity. Respiratory frequency was increased and tidal volume was reduced. Airway conductance at resting lung volume was not changed despite reduced functional residual capacity. There was slight unevenness of alveolar ventilation with evidence of non- or poorly ventilated lung units. Systemic arterial oxygen tension fell during oxygen breathing, and there was a slight increase of peripheral venous pressure. Following release of chest restriction, the mechanical changes in the lungs were reversed via a hysteresis-like pathway. They appear to be explained in part by the opening of lung units which had become closed during chest restriction.
—E. DUNNER

Physiological Factors Affecting Airway Resistance in Normal Subjects and in Patients with Obstructive Respiratory Disease. J. BUTLER*, C. G. CABO, R. ALCALA, and A. B. DUBOIS. *J Clin Invest*, April, 1960, 39: 584–591. (*Graduate School of Med., U. of Pennsylvania, Philadelphia)

The effects on airway conductance (the reciprocal of airway resistance) of chest strapping, bronchomotor drugs, exercise, forced breathing, oxygen, carbon dioxide, and old age, were measured in normal subjects and compared with similar observations in patients with asthma, bronchitis, and emphysema. Airway conductance was found to be dependent upon lung elastic pressure rather than on lung volume. This relationship was altered by bronchomotor drugs. Breathing oxygen and different concentrations of carbon dioxide did not alter the airway resistance. In several patients with mild asthma, airway resistance increased after forced breathing or exercise. In normal subjects, airway conductance appeared to depend upon a balance of forces between the tension in the airway walls and the opposing transmural pressure gradient due to the elastic traction of the lung. The airway conductance was found to be reduced and the balance of forces was altered in patients who had lower airway obstruction.
—E. DUNNER

Hypertensive Pulmonary Arterial Lesions as a Late Result of Anastomosis of Systemic and Pulmonary Circulations. C. A. WAGENVOORT*, J. W. DUSHANE, and J. E. EDWARDS. *Proc Mayo Clin*, April 13, 1960, 35: 186–192. (*Mayo Clinic, Rochester, Minn.)

A boy who died at the age of 8 years had a cardiac malformation manifested by an origin of both the aorta and the pulmonary artery from the right ventricle and by pulmonary stenosis. Potts and Blalock anastomoses had been done at the ages of 1½ and 2 years, respectively; these shunt operations were followed by pulmonary hypertension. Lesions in the pulmonary arterial branches were partly identical with those seen in pulmonary hypertension and were considered to result from altered flow-pressure relationships accompanying the created shunts. Some of the lesions were like those found in pulmonary steno-

sis; these were thought to have existed from the period prior to the creation of the shunts (Authors' summary).—E. A. Riley

The Polymask as a Means of Administering Oxygen. M. Catterall* and M. Snow. *Brit Med J*, April 23, 1960, No. 5181: 1254–1256. (*General Infirmary, Leeds, England)

The delivery of oxygen to a patient through the polymask is unpredictable whatever the rate of flow of oxygen from the cylinder, but the higher the rate of flow the more chance there is of an oxygen-rich atmosphere being available to the patient. From the evidence of gas analyses, the mask is inefficient and the wearer is more likely to breathe room air than oxygen-rich atmosphere. There is no undue accumulation of carbon dioxide (Authors' summary).—E. A. Riley

A Study of the Repeatability of Ventilatory Tests, Anthropometric Measurements, and Answers to a Respiratory Symptoms Questionnaire in Working Coal Miners. J. R. Ashford*, G. D. Forwell, and R. Routledge. *Brit J Industr Med*, April, 1960, 17: 114–121. (*Pneumoconiosis Field Research of the National Coal Board, England)

A study has been made in working coal miners of the repeatability of ventilatory tests, anthropometric measurements, and the answers to a questionnaire on respiratory symptoms. On the first visit, especially, there was evidence of a continuous increase in both forced expiratory volume (FEV) and forced vital capacity (FVC) from the first to sixth expirations at the same visit, but this was minimal after the first expiration. The effect on the FEV and FVC readings of the day of the week and time of day at which the examination was made was negligible. The observations recorded in Scotland were 15 per cent higher than those obtained in South Wales. Between the beginning and end of the working shift, there was a small reduction in weight and in standing and sitting height measurements. The repeatability of the answers to the questionnaire was good. No consistent difference was found between doctors and clerical observers (after authors' summary).—H. J. Simon

A Study of the Reproducibility of the Forced Vital Capacity of Coalworkers at Two Collieries in Scotland and Two Collieries in South Wales. D. P. Duffield* and J. R. Ashford. *Brit J Industr Med*, April, 1960, 17: 122–124. (*National Coal Board, Pneumoconiosis Field Research, England)

A study of 1,522 coal workers at 2 Scottish and 2 Welsh collieries showed that the forced vital capacity (FVC) was as reproducible a measurement as was the forced expiratory volume (FEV) (1-second). This applied not only to replicate readings, but also to readings taken before and after a working shift on the same day, and to readings taken at an interval of 1 week. An examination of the results of FEV (1-second) and FVC readings taken of the entire colliery populations at a colliery in Ayrshire, Scotland, and at 1 in South Wales, showed that replicate readings of FVC and FEV (1-second) were equally reproducible, and that reproducibility was virtually unaffected by the presence or absence of bronchitic symptoms or by the differing prevalence of pneumoconiosis at the 2 collieries (after authors' summary).—H. J. Simon

MICROBIOLOGY AND IMMUNOLOGY

Characteristics of a Strain of Acid-fast Bacilli Isolated from an Osteomyelitic Lesion Which Developed after BCG Vaccination (in Japanese). T. Morioka*. *Kekkaku*, May, 1960, 35: 331–337. (*Keio U., Tokyo, Japan)

A strain of acid-fast bacilli was isolated from a female patient with tuberculous lesions of the skin and bones after BCG vaccination. The patient received the vaccination at the age of 1 year and 4 months, and an ulcer formed at the inoculated site. About 1 year later, spina ventosa of the left ring finger and, 1 year thereafter, osteomyelitis of the left tibia and fibula developed. She was treated with chemotherapy and surgery. A strain of acid-fast facilli was recovered from one of the surgical specimens and the characteristics of the strain were as follows: (*1*) It was acid fast, with a Kf value greater than 10, and it formed feeble cord, as did the control BCG strain. (*2*) The strain was not resistant to streptomycin, isoniazid, and PAS, but was resistant to pyrazinamide. The same degree of resistance to the latter was observed in all of the control strains of bovine tubercle bacilli, including the BCG strain, but not in the control strains of human tubercle bacilli. (*3*) It stained faintly with neutral red, similar to BCG, but not as strongly as virulent tubercle bacilli. (*4*) In the niacin test of Konno, this strain showed results similar to the bovine strain. (*5*) It grew very weakly in Besredka's medium, in contrast to all of the strains of tubercle bacilli other than BCG, which grew abundantly. (*6*) After intravenous inoculation in guinea pigs, this strain decreased gradually in number *in vivo*, as did the BCG strain. It was nonpathogenic on intracranial inoculation, and no lymphadenopathy developed on sub-

cutaneous injection. The writer concluded that all of the characteristics described above favored the conclusion, with reservations, that the isolated strain was an attenuated strain of bovine tubercle bacilli.—I. TATENO

Comparative Study of the BCG Test Performed in Man with Isoniazid-susceptible and -resistant Bacilli (in French). A. TACQUET*, B. GAUDIER, and J.-P. PECQUEREAUX. *Ann Inst Pasteur Lille*, 1958–59, 10: 51–60. (*National Institute of Hygiene, Paris, France)

This is a comparative study of skin tests performed with isoniazid-susceptible and -resistant BCG. Scarifications performed with heat-killed vaccine both susceptible and resistant to the drug gave similar results in tuberculous patients treated with the drug. Intradermal reactions performed with resistant BCG were more intense and appeared earlier than did those obtained with isoniazid-susceptible BCG. Steroids completely inhibited the response to scarification with BCG and attenuated the development of 50 per cent of intradermal BCG skin tests. Scarifications and intradermal reactions performed with living susceptible and resistant BCG in nontuberculous patients gave similar responses. Two strains, resistant to 10 and 100γ, respectively, have been used in these series. No difference was noted in the results with either strain.—V. LEITES

Antifungal Drug in Tubercle Bacillus Cultures (in German). W. BREHMER* and G. MOLL. *Beitr Klin Tuberk*, November, 1959, 121: 393–397. (*Robert Koch Institut, Berlin, Germany)

Contamination of tuberculosis cultures by fungi can be prevented by adding a polyene antifungal agent to the medium (nystatin, isolated from liquid cultures of *Streptomyces noursei*, was used). The growth of human and bovine cultures are not affected by the drug; however, it is inactivated by egg-containing media.—Z. VIRÁGH

Method of Niacin Determination in Cultures of Mycobacteria (in Spanish). B. MEDINA*. *Rev Lat Amer Microbiol*, December, 1959, 2: 227–236. (*Unidad de Patologia, Escuela de Medicina, México)

This modification of Konno's niacin test employs cultures grown in liquid media. Cyanogen bromide and *o*-tolidine are used as reagents. All of the human strains tested were positive; one of the bovine strains was weakly positive. Among the rapid-growing bacilli, the pigment-producing strains were negative, and the nonpigmented strains were positive. The niacin content was greater in older cultures. The best medium for niacin production was Proskauer and Beck's with 2 per cent glycerol. The niacin content decreased when glucose was substituted for glycerol or when the glycerol was used in higher concentration.—Z. VIRÁGH

Viability of the H37Rv Strain of *M. Tuberculosis* in Bovine Plasma Albumin and Charcoal Water Diluents after Varying Periods of Exposure at Room and Ocebox Temperatures: A Note. M. S. TARSHIS*. *Acta Tuberc Scand*, No. 1, 1960, 39: 76–79. (*V.A. Hosp., Alexandria, La.)

In a comparative study of the viability of the tubercle bacillus in diluents after varying periods at room and icebox temperatures, it was found that the H37Rv strain of *M. tuberculosis* was capable of surviving in both plasma albumin and charcoal water diluents either at room temperature or 5°C. for as long as 22 days. With increase in the exposure time of the organism in the diluents there was a corresponding decrease in viability. After the first 2 days of exposure in both diluents, viability was slightly greater at 5°C. than at room temperature. After 3 days of exposure, with 1 exception, the reverse was noted, viability being greater at room temperature than at 5°C.; this effect was slight from the 3rd through the 7th days and significantly greater from the 15th through the 22nd days. With only 3 exceptions, survival of the organism in plasma albumin was greater than in charcoal water at both temperatures after all periods of exposure. Treatment of the organism by these procedures resulted in no significant alterations either in morphology, staining, or virulence.—W. J. STEININGER

A Study of Nitrate Reduction by Mycobacteria. The Use of the Nitrate Reduction Test in the Identification of Mycobacteria. S. VIRTANEN*. *Acta Tuberc Scand*, 1960, 38: Supp. 48, 1–119. (*U. of Turku, Finland)

This monograph deals with the nitrate reduction test developed by the author and its applicability to the typing of mycobacteria and to the investigation of their metabolism. It was found that the nitrate reduction test can be used for the differentiation of *M. bovis*, BCG, and BCG-like unclassified human strains from typical human strains of *M. tuberculosis*. Moreover, a considerable proportion of *M. avium* and unclassified acid-fast organisms can be distinguished from the typical human strains. Strains which give a negative or very weak nitrate reduction reaction are not typical human strains of *M. tuberculosis*. Biochemical tests are complementary to other investigative procedures in the classification of mycobacteria.—W. J. STEININGER

EXPERIMENTAL PATHOLOGY

Revaccination with BCG Vaccine. An Experimental Study in Guinea Pigs. M. MAGNUSSON*, A. JESPERSEN, and M. W. BENTZON. *Acta Tuberc Scand*, No. 1, 1960, 39: 34–56. (*Statens Seruminstitut, Copenhagen, Denmark)

In an experimental study in guinea pigs, investigation was made of the practice of recommending the revaccination of humans with BCG vaccine when the tuberculin sensitivity after primary vaccination with BCG can no longer be demonstrated with 5 TU of tuberculin. Groups of guinea pigs were vaccinated intracutaneously with BCG vaccine at various times; after a suitable period, some of the animals were revaccinated with BCG. When challenged with virulent bovine tubercle bacilli, all of the groups of vaccinated animals had longer average survival times than did the nonvaccinated controls. Nine months after the primary vaccination, tuberculin sensitivity had waned very considerably, but the immunity was the same as that possessed by animals vaccinated 2 months before challenge. By means of tuberculin tests carried out during the vaccination period, the waning in tuberculin sensitivity could almost be avoided without immunity being affected. Revaccination increased the immunity and induced slightly less tuberculin sensitivity than did the primary vaccination. It is concluded that revaccination with BCG is justified as soon as possible after the tuberculin sensitivity induced by primary vaccination has waned, although it is perhaps not absolutely necessary.—W. J. STEININGER

Study of Animal Pathogenicity of Atypical (Unclassified) Mycobacteria. The Effect of Influenza Virus and of Quartz upon Experimental Infection (in French). C. GERNEZ-RIEUX*, A. TACQUET, and V. MACQUET. *Ann Inst Pasteur Lille*, 1958–59, 10: 9–28. (*Institut Pasteur, Lille, France)

Eighty cultures of unclassified mycobacteria have been isolated from 40 patients, 10 of whom had coal miners' pneumonoconiosis; 22 of the cultures belonged to the photochromogenic group, 22 to the scotochromogenic, and 36 to the nonphotochromogenic group. The pathogenicity of these bacteria was studied in guinea pigs, mice, hamsters, rabbits, and hens. Intraperitoneal inoculation of these mycobacteria and of influenza virus does not show increased virulence for guinea pigs. Guinea pigs inoculated intratracheally with a mixture of quartz and photochromogenic or nonphotochromogenic bacteria develop much more severe lesions than do animals inoculated only with quartz or mycobacteria (after authors' summary).—V. LEITES

Skin Sensitisation of Guinea-Pigs with Atypical Acid-Fast Bacilli. A. BECK*. *J Path Bact*, April, 1960, 79: 285–288. (*Paddington General Hosp., London, England)

Quantitative skin sensitivity tests, with protein fractions of 4 strains of unclassified acid-fast bacilli and of other mycobacteria, in guinea pigs infected with these bacilli revealed marked cross-reactivity of these antigens. A quantitative evaluation of these tests suggests that unclassified acid-fast bacilli form a serologic group which can be distinguished from both tubercle bacilli and saprophytic mycobacteria (after author's summary).—H. J. HENDERSON

Allergic Reactions Produced by Isoniazid-resistant and -susceptible Tubercle Bacilli (in German). T. FODOR*. *Beitr Klin Tuberk*, December, 1959, 121: 455–459. (*Pihenő-ut 1, Budapest, Hungary)

Thirty guinea pigs were first immunized by subcutaneous injection of 0.5 mg. vole bacillus culture. After 6 weeks, they were inoculated with 0.001 mg. of H37Rv culture. Ten healthy animals served as controls. To provoke an allergic reaction, the immunized guinea pigs were again inoculated: some were given susceptible bacilli, another group received resistant bacilli, and the last 10 animals were given avirulent H37Ra bacilli. The allergic reaction was similar in all cases. The conclusion is that isoniazid-resistant tubercle bacilli—believed to be less pathogenic—are capable of provoking allergic reactions which, in turn, may cause exacerbation in a patient with inactive disease.—Z. VIRÁGH

Serum Glycoproteins in Tuberculous Patients (in German). L. LEVENDEL*, E. KÁNITZ, and T. ERDÖS. *Beitr Klin Tuberk*, December, 1959, 121: 447–450. (*Korányi-Landesinstitut für Tuberkulose, Budapest, Hungary)

More than 300 tests were done in 146 patients with pulmonary tuberculosis; 97 healthy persons served as controls. The serum glycoprotein level was significantly elevated in the patients; however, this increase is not specific for tuberculosis. —Z. VIRÁGH

Passive Transfer of Resistance to Tuberculosis through Use of Monocytes. J. L. SEVER*. *Proc Soc Exp Biol Med*, February, 1960, 103: 326–329. (*Northwestern U., Chicago, Ill.)

A limited degree of resistance to tuberculosis in mice of the Strong A strain was obtained after the transfer of monocytes from immunized mice. The passive transfer of monocytes from normal animals had no effect on resistance. No increased resistance was seen in mice receiving plasma, spleen

homogenate, or spleen filtrate obtained from either normal or immunized animals.—E. S. LEAKE

The Action of Tuberculin on the Osmotic Resistance of the Leukocytes of Guinea Pigs in the Course of Experimental Tuberculous Infection (in Italian). D. ZANNONI* and L. ABATE. *Riv Pat Clin Tuberc*, No. 5, 1959, 32: 573–581. (*Ist. C. A. Pizzardi, Bologna, Italy)

Lysis of the leukocytes of guinea pigs experimentally infected with tubercle bacilli was studied by considering the variations in their resistance against hypotonic and normal saline solutions containing tuberculin. In the infected animals, a sharp increase was demonstrated in the osmotic lability of the leukocytes against saline solutions containing tuberculin. This increase was more evident in the polynuclears than in the mononuclears.—G. CASTELNUOVO

SYMPOSIA

The Principal Reports to the Convention of the Swiss Antituberculosis Association Held in Geneva on May 2–3, 1959. *Schweiz Z Tuberk*, No. 6, 1959, 16: pp. 363–414.

Chronic Bronchitis (in German). P. ZUIDEMA*. Pp. 363–382. (*Nederlands Sanatorium, Davos, Switzerland)

The semeiology, etiology, bacteriology, diagnostic procedures, and the various forms of the disorder are described.

Pulmonary Function in Chronic Bronchitis (in French). P. ROSSIER*. Pp. 382–392. (*Clinique Médicale de l'Université, Zurich, Switzerland)

There are two forms of chronic bronchitis: one affects the large and medium-sized bronchi, and the other, the small bronchi and bronchioli. The former is encountered in adults—especially in smokers—with only functional symptoms, without dyspnea or emphysema. Cough and expectoration are present as a sign of irritation, but the pulmonary function is not impaired. If the disorder affects the small bronchi and bronchioli, the syndrome of obstructive bronchitis or bronchial stenosis develops. In the former, the ensuing dyspnea and hypoventilation are the causes of hypoxemia and hypercapnia, which conditions, together with the increased vascular resistance, maintain hypertension in the lesser circulation: the preceding stage of the complex disorder called "cor pulmonale." Bronchial stenosis leads to the same condition through the development of obstructive emphysema which, by causing atrophy of the alveolar lining with a decrease in the pulmonary capillary bed, also produces pulmonary hypertension.

Etiologic Factors in Chronic Bronchitis (in French). N. OSWALD*. Pp. 392–396. (*70 Harley St., London, England)

On the basis of statistical data from Great Britain and Wales, it is believed that the most important factors in the etiology of chronic bronchitis are: heredity, age, social situation, occupation, climate, and the degree of air pollution. Infection is also an essential factor.

Bronchitis-Bronchiolitis (in German). M. KARTAGENER*. Pp. 396–399. (*Börsenstrasse 18, Zürich 1, Switzerland)

The term "chronic bronchitis" usually denotes a noninflammatory condition characterized by the findings of obstructive emphysema caused by either an allergic spasm of the bronchial muscles or inflammatory changes in the bronchioli. True chronic bronchitis—that is, the inflammation of the primary and secondary bronchi—is a rare disorder which practically never terminates fatally. On the other hand, chronic bronchiolitis—with or without obliteration—is rather frequent; its treatment is almost identical to the prophylaxis of emphysema.

Endoscopy in Chronic Bronchitis (in French). J. LEMOINE*. Pp. 400–404. (*189 St. Germain, Paris, France)

Of 131 cases examined by bronchoscopy, tracheitis with diffuse bronchitis was diagnosed in 31, tracheitis with circumscript bronchitis in 11, diffuse bronchitis in 44, and circumscribed bronchitis in 45 patients. The trachea does not always participate in the inflammatory processes; isolated tracheitis is rare—it was not diagnosed in a single case among the 131.

Chronic Bronchitis (in Italian). N. MONTANINI*. Pp. 404–409. (*Via Crescenzio 19, Rome, Italy)

Attention is directed to the necessity of scientifically precise definition and classification of the entity, chronic bronchitis. It is believed that there are many asymptomatic cases of chronic bronchitis. Arriving at the diagnosis by way of bronchoscopy, bronchography, and even biopsy, is important, especially in the knowledge that sometimes healed miliary tuberculosis or some other pathologic conditions may be the underlying causes of the symptoms.

Surgical Importance of Chronic Bronchitis (in German). W. BRUNNER*. Pp. 410–414. (*Bergstrasse 48, Zurich, Switzerland)

A frequent cause of the postoperative complications in thoracic surgery—even in upper gastrointestinal operations—can be chronic bronchitis. Anesthetics may cause severe bronchial spasm in

patients with asthmatic bronchitis at the beginning of or during the operation. Postoperatively, patients with bronchial asthma or chronic bronchitis may develop a subileus by passively swallowing a large amount of air. Postoperative atelectasis, subcutaneous or mediastinal emphysema, and bronchial fistulas are observed mostly in patients with chronic bronchitis; consequently, if this condition is not of long standing and a cure is likely, it should be treated before the operation is performed. Of the various palliative surgical procedures intended to relieve the symptoms of chronic, asthmatic, or emphysematous bronchitis, only the excision of compressive emphysematous bullae is justified.—Z. VIRÁGH

PUBLIC HEALTH AND EPIDEMIOLOGY

Pulmonary Asbestosis (in Spanish). P. SCHÜLER*, V. MATURANA, E. CRUZ, C. GUIJÓN, A. VALENZUELA, H. OYANGUREN, R. HADDAD, and D. BENQUIS. *Rev Chil Enferm Toraz*, No. 25, 1959: 37–53. (*Dept. de Higiene Industrial y Med. del Trabajo, Chile)

Of 319 workers employed in asbestos processing industries, 158 (49 per cent) were healthy, 106 (33 per cent) were suspected to have, and 55 (17 per cent) were diagnosed to have, asbestosis. The average time of exposure was 17.6 years. Of the 55 patients, only 12 were asymptomatic; the rest presented symptoms of cough, mucopurulent expectoration, and dyspnea. Roentgenograms revealed evidence of pulmonary fibrosis, increased bronchial markings, nodules and densities of various sizes, and emphysema. Asbestos particles were demonstrated in the sputum of 95 per cent of the patients. None of these patients had tuberculosis or carcinoma associated with the disease. —Z. VIRÁGH

The Occurrence of Cancer in Husbands and Wives. F. A. NASH*. *Brit J Cancer*, December, 1959, 13: 576–588. (*Western Hosp., Fulham, London, England)

In an investigation of shared domestic environmental factors in the etiology of cancer, study was made of the causes of death of the husbands of 417 widows who died from cancer ("cancer widows") and of a control group of 455 widows who died of causes other than cancer ("non-cancer widows"). It was found that 20 per cent of the husbands of "cancer widows" died from cancer. The percentage of husbands of "non-cancer widows" who died of cancer was 20.6 per cent. There is no evidence from the present investigation that domestic factors or habits common to husband and wife are carcinogenic.
—W. J. STEININGER

The Increase in Lung Cancer Mortality in Canada. A. J. PHILLIPS*. *Brit J Cancer*, December, 1959, 13: 589–594. (*National Cancer Institute of Canada, Toronto, Ontario)

In a review of deaths in Canada attributed to the major diseases of the respiratory system during the years 1931 to 1956, it was found that lung cancer has increased approximately 8 times in males and has doubled in females. Significant declines for each sex were found in deaths from tuberculosis and from all other respiratory diseases. The assumption that all of the increase in mortality attributed to lung cancer since 1931 could be accounted for by erroneous death certification to other respiratory diseases, including tuberculosis, during the earlier years of the period cannot be substantiated without unreasonable assumptions of age and sex differences in diagnostic error. It is shown that a substantial proportion of the recorded increase can be accounted for theoretically by a diagnostic error of 5 per cent. Thus, if 5 per cent of the deaths attributed to respiratory diseases including tuberculosis were actually due to lung cancer, the recorded increase in males since 1931 in Canada would be 3.6 times instead of 8.2 times, and in females no increase would be evident. This increase would be reduced somewhat further on the assumption of greater diagnostic error in the earlier than in the later years under review.—W. J. STEININGER

Peptic Ulcer and Pulmonary Tuberculosis (in German). A. ENTZ*, J. MÁRK, and G. RÓKA. *Tuberkulosearzt*, December, 1959, 13: 834–839. (*Pihenő-út 1, Budapest, Hungary)

The incidence of peptic ulcer in 1,105 patients with pulmonary tuberculosis was 5.6 per cent (62 cases). Gastrectomy was performed in 36 cases (3.25 per cent), while the remaining 26 were treated conservatively. An analysis of the data indicates that an ulcer does not predispose to tuberculosis, but that the altered tone of the autonomic nervous system caused by tuberculosis predisposes to the development of ulcer.
—J. HAAPANEN

Calcifying Lymphadenitis Following BCG Vaccination (in German). FR. HAGGENMÜLLER*. *Tuberkulosearzt*, December, 1959, 13: 819–822. (*Kinderklinik, Augsburg, Germany)

Calcification of the axillary lymph nodes was observed in a 3-year-old girl who had undergone BCG vaccination shortly after birth. It is suggested that a calcifying lymphadenitis after BCG vaccination is not as rare as may seem from the paucity of reported cases.—J. HAAPANEN

The Medical and Social Incidence of Resistance of Koch (Tubercle) Bacilli to Antimicrobial Drugs (in French). A. MEYER. *J Pneum-Phtis Afrique Nord*, Nos. 3–4, 1959: 91–101.

The medical and social aspects of drug resistance are discussed. The three conventional drugs (isoniazid, PAS, and streptomycin) are taken into consideration. The medical problems arising from resistance are given in detail, especially in connection with surgery and recurring disease. The possibility of infection in contacts with drug-resistant bacilli presents a special problem in some countries due to the prevailing social conditions. These are the countries where there are not sufficient hospital facilities to take care of all of the active cases of tuberculosis and, also, where the patients do not accept hospitalization readily. Thus they have to be treated as outpatients, and in many cases the patients do not follow the ordered regimen faithfully. On the other hand, they live in close quarters with many people crowded into one room, and infection is inevitable. No statistics are given on the frequency of drug resistance in newly infected cases.—E. LYON

Observations on the Apparently Recovered Patient with Cavitary Tuberculosis as an Ignored Source of Contagion (in Italian). E. MARINO*. *Lotta Tuberc*, No. 12, 1959, 29: 1768–1775. (*U. of Siena, Italy)

This study was undertaken to ascertain how often an apparently recovered tuberculous patient may become a source of spread of tubercle bacilli into his household and community. Twenty-two persons, 19 to 63 years old, with cavitary tuberculosis of the lung, who went back to their families and often to their work because they seemed to be fully recovered after the use of antimicrobials, were followed up again a few months after they had been dismissed from the hospital: 4 of 22 (18 per cent) were found to have sputum positive for *M. tuberculosis*. Of 144 persons belonging to the 22 households, 7 were found to have active tuberculous processes, and their close relationship with the 4 sputum-positive patients was established.—I. ARCHETTI

Familial Sarcoidosis: Epidemiological Aspects with Notes on a Possible Relationship to the Chewing of Pine Pitch. R. B. BAER*. *AMA Arch Intern Med*, January, 1960, 105: 60–68. (*300 Pasteur Drive, Palo Alto, Calif.)

In a remarkable family of 12 siblings, 4 members have sarcoidosis (and possibly a fifth), making this the third reported family having more than 2 members with sarcoidosis. The group grew up in a rural area surrounding Pineland, Texas. In childhood, the 5 involved members chewed heavily a modified pine pitch which in the springtime included "pine buzz" (undoubtedly pine pollen). A gastrointestinal portal of entry of pine pollen is suggested, and evidence that sarcoid granulomas may originate from the stomach is cited. It is hoped that other investigators closer to the sarcoid "area" will give further thought to the possibility that gastrointestinal absorption of pine tree products is related to sarcoidosis.
—W. J. STEININGER

The Epidemiology of Primary Atypical Pneumonia. H. B. HARDING* and R. A. SNYDER. *AMA Arch Intern Med*, February, 1960, 105: 217–232. (*Northwestern U. Med. School, Chicago, Ill.)

Experience with 116 cases of epidemic and sporadic primary atypical pneumonia is presented. Evidence for the possible existence of a subclinical type of the disease also is given. It again has been found that primary atypical pneumonia apparently is not due to any of the common bacteria nor the better known viruses or rickettsiae. The sporadic type of the disease has been observed more often in the late summer and fall than at other times of the year, and more often in females than in males. The literature concerning the disease is reviewed extensively, including the techniques for and the mechanism of the cold hemagglutinin and streptococcal MG agglutinin tests (Authors' summary).—W. J. STEININGER

Report on the Nationwide Occurrence of Q Fever Infections in Cattle. L. LUOTO*. *Public Health Rep*, February, 1960, 75: 135–140. (*Rocky Mt. Lab., U.S.P.H.S., Hamilton, Montana)

Q fever in bovines, present in only 7 states in 1949, is now reported in 35 states. Human disease contracted from livestock is already a public health problem in some areas and is being recognized in others previously considered to be free from infection. Concerted nationwide studies of the occurrence and epidemiology of the disease are needed to define factors bearing on its occurrence and spread.—E. DUNNER

Approach to Zero for Tuberculosis. Conference Report. *Public Health Rep*, February, 1960, 75: 103–106.

Morbidity and Mortality Characteristics of Asian Strain Influenza. T. D. Y. CHIN*, J. F. FOLEY, I. L. DOTO, C. R. GRAVELLE, and J. WESTON. *Public Health Rep*, February, 1960, 75: 149–158. (*Kansas City Field Station, U.S.P.H.S., Kansas)

Asian influenza appeared in epidemic prevalence in the greater Kansas City area during the fall of 1957, followed by a minor secondary wave during

the winter of 1958. A survey of 5,822 persons during the fall epidemic revealed an attack rate of 34 per cent. The highest attack rate was observed in persons aged 10–19 years, with a decline in rates with increasing age. A total of 253 deaths due to influenza and pneumonia was reported in Kansas City, Missouri, from October 1, 1957, to March 31, 1958, a mortality rate of 49.6 per 100,000. This rate was 2 to 3 times higher than that of the 4 preceding noninfluenza years. Two peaks were observed in the mortality curve, one occurring in October and November, and the secondary peak in the latter part of February. The highest death rates were observed in the very young and the very old. Seventy-three per cent of the persons who died had a history of pre-existing chronic disease or other associated conditions, the majority of which were listed as cardiovascular or chronic pulmonary disease.—E. DUNNER

Pilot Histoplasmosis Survey in Delhi Area. R. VISWANATHAN*, S. C. CHAKRAVARTY, H. S. RANDHAWA, and A. J. H. DE MONTE. *Brit Med J*, February 6, 1960, No. 5170: 399–400. (*U. of Delhi, India)

A pilot histoplasmin survey was carried out in the Delhi area. Skin tests were performed on 747 people from different places representing rural, urban, and riverine areas of Delhi: 12.3 per cent positive reactors were found in the riverine area, 2.9 per cent in the rural area, and 4.9 per cent in the urban area. Cultural and serologic examinations of histoplasmin-positive reactors were all negative. (Authors' summary).—E. A. RILEY

Cigarette Smoking and Lung Cancer in Canada. E. C. HAMMOND* and E. L. WYNDER. *Canad Med Ass J*, February 15, 1960, 82: 372–378. (*American Cancer Society and Sloan Kettering Institute, N.Y., N.Y.)

A total of 6,428 discarded cigarette butts was collected and measured in Montreal, Toronto, Vancouver, and Hamilton. They were classified by type and length of cigarette, location, socioeconomic class, and sex of the smoker. The mean length of all the butts was 27.9 mm. This is slightly less than the reported mean length of cigarette butts collected in the United States (30.9 mm.), but much greater than the mean length of butts collected in England and Wales (18.7 mm.) and in the Netherlands (20.8 mm.). The mean length of the cigarette butts was greater for those collected from the upper socioeconomic classes, intermediate for those collected from the middle classes, and least for those collected from the lower classes. Of the butts collected from the upper classes, 59.4 per cent had filter tips; of those collected from the middle classes, 61.3 per cent had filter tips; and of those collected from the lower classes, 44.4 per cent had filter tips. The mean length of butts discarded by women did not differ significantly from the mean length of those discarded by men. There was also little difference between men and women in respect to the percentage of filter-tip cigarettes smoked. In general, the butts from filtered cigarettes were slightly longer than those from regular cigarettes, although the difference was not statistically significant. The average amount of tobacco consumed per cigarette appears to be roughly proportional to the original length of the cigarette. Smoke from the first part of a cigarette contains proportionally less tar than smoke from the latter part of a cigarette. Therefore, the dosage of tar increases rapidly with the amount of a cigarette consumed. For this reason, it is suggested that average butt length be taken into consideration when estimates are made of the average exposure of various population groups to cigarette smoke. The differences in average butt length may partially account for reported differences in lung cancer death rates in various countries and various socioeconomic classes (after authors' summary).—E. A. RILEY

Smoking and Lung Cancer. Editorial. *New Engl J Med*, February 25, 1960, 262: 417–418.

It is more than 20 years since Drs. Lombard and Doering, in this journal, first suggested an association between smoking and cancer. Since that time there have been more than two score similar studies, all of which have shown the same association and have progressively implicated excessive cigarette smoking. No responsible observer can deny this association, and the evidence is now sufficiently strong to suggest a causative role. In the face of this evidence, the response of the tobacco manufacturers and their spokesmen has been deplorable. They have been unwilling to follow the example of the distilling industry, which reaps profits while never denying—and occasionally calling attention to the fact—that excessive drinking leads to drunkenness. It is true that much remains to be learned about the pathogenesis of lung cancer and that smoking is certainly not the only cause. However, it is not necessary to have precise information on etiology to prevent disease. Vaccination protected against smallpox more than 100 years before the virus was identified, and *Vibrio cholerae* was unsuspected at the time that John Snow had the foresight and courage to take the handle off the Broad Street pump. Lives will continue to be lost if control measures must await definitive studies. Lives will be saved if physicians can now persuade their patients to stop smoking.—M. J. SMALL

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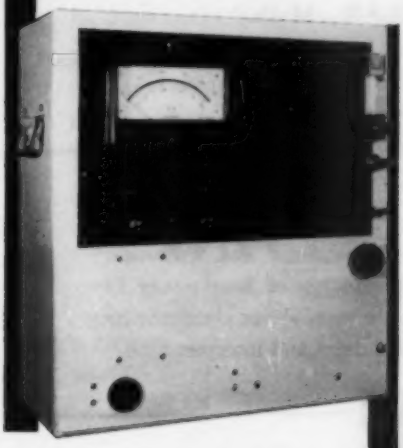

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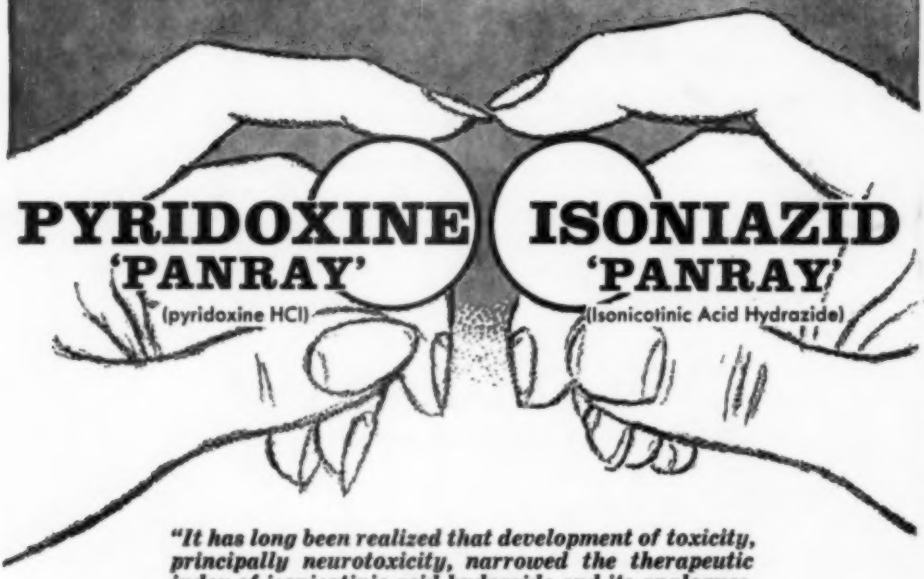

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[1]See View Bulletin 16:62 July '56 Tchertkoff, et al. JAMA 156:1549 (Dec. 25) 1954 Biehl & Vilmer Am. Rev. Tbc 70:266 Aug. '54 Hughes, et al. N. Eng. J. of Med. 255 (3 118-122, 1956 Carlson, et al.